Towards Patient-Tailored Care for Soft Tissue Sarcoma

IBTISSAM ACEM

Stellingen behorende bij het proefschrift

Towards Patient-Tailored Care for Soft Tissue Sarcoma

1. Prediction tools facilitate a shift from a one-size-fits-all approach to patient-tailored management of soft tissue sarcoma. (this thesis)

2. Prediction tools should be recalibrated with setting-specific estimates. (this thesis)

3. Novel minimal-invasive diagnostic tools in cancer should focus on maximizing sensitivity rather than specificity. (this thesis)

4. Neoadjuvant chemotherapy should be offered in a selected group of high-risk patients with a soft tissue sarcoma of the extremities. (this thesis)

5. All patients with a soft tissue sarcoma should be restaged after neoadjuvant radiotherapy. (this thesis)

6. All PhD'ers dealing with time-to-event data and missing values should learn R.

7. Prediction models relying on observational data should not be used to estimate individual survival benefits of various adjuvant therapy options included in the model.

8. Internal-external validation procedures should be used at the time of model development rather than keeping parts of the data out for external validation. (Ewout W. Steyerberg, J Clin Epidemiol. 2016)

9. Because causal inference from observational data can be viewed as an attempt to emulate a target trial, the question is not whether observational data should be used for causal inference, but rather how to use them most effectively. (Miguel A. Hernán, N Engl J Med. 2021)

10. Het perfecte is de vijand van het goede. (Pensées, Montesquiue)

11. "Most epidemiologists don't study epidemics?"

Ibtissam Acem

TOWARDS PATIENT-TAILORED CARE FOR SOFT TISSUE SARCOMA

Ibtissam Acem

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TOWARDS PATIENT-TAILORED CARE FOR SOFT TISSUE SARCOMA

Op weg naar gepersonaliseerde zorg voor
patienten met weke delen sarcomen

Proefschrift

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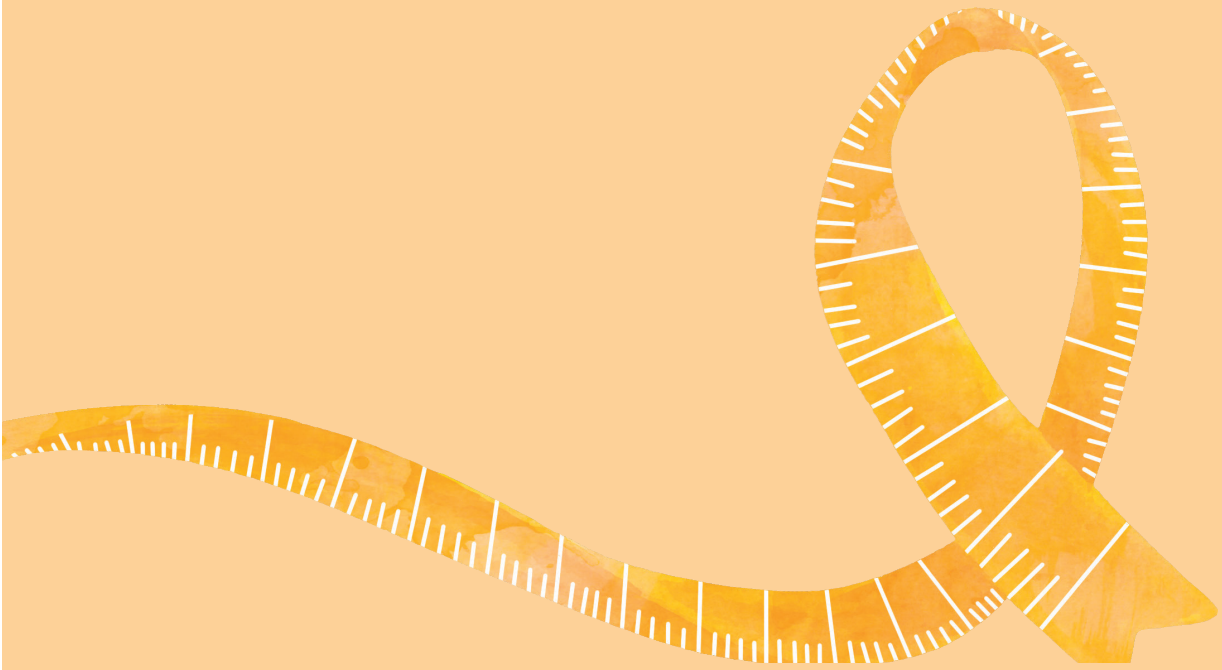
Chapter 1

General Introduction and Thesis Outline



Epidemiology of STS

Soft tissue sarcomas (STS) represent a group of rare and heterogeneous malignant neoplasms arising from mesenchymal cells. (1) STSs encompass over 100 different histological subtypes and account for 1% of malignancies in adults, 10% in children and 8% in adolescents and young adults (AYAs, 18-39 years of age). (1, 2) Annually, 700-800 new patients are diagnosed with STS in the Netherlands. (3) STSs may occur at any age and are one of the most common type of cancers in children, adolescents and young adults. (4) The incidence of STS increases with age, with an average age of 61 years. (5, 6) STS arise from all types of soft tissue, such as nerve sheaths, blood vessels, muscles, fat and tendons. (1) The mostly affected sites are the extremities and trunk wall. (5, 6) The most common histological subtypes are leiomyosarcoma, undifferentiated pleomorphic sarcoma and liposarcoma. (6) Approximately 10-15% of the patients present with metastatic disease at time of diagnosis and around 30% of the patients with primary high-grade STS develop metastatic disease within five years after primary treatment. (6-8) STS mainly metastasize to the lungs. (9, 10)

Presentation and diagnostic work-up

Soft tissue sarcomas of the extremities (eSTS) often present as unspecific and painless lumps (11), which make diagnosing eSTS challenging among the large number of benign soft tissue tumours. It has been estimated that benign soft tissue tumours occur 300 times more often than their malignant counterparts. (12, 13) Therefore, patients with eSTS are at risk of delayed or incorrect diagnosis. (14) In a nationwide survey in England, 1 in 4 sarcoma patients reported that they waited more than 3 months after first symptoms before visiting a medical professional. (11) Besides this patient delay, in 28% of the sarcoma patients an incorrect interpretation of the symptoms and subsequent advice was given. (11) A nationwide survey in the Netherlands reported that in 28% of the patients the diagnostic trajectory took more than 3 months after first consultation with a physician. (15) AYAs are most likely to receive an incorrect diagnosis compared to older patients. (11)

Given the rarity and the unspecific presentation of STS, unplanned excisions without appropriate diagnosis, preoperative imaging and planning are frequently performed. (13, 16-21) In primary soft tissue tumours of the extremities, magnetic resonance imaging (MRI) is the main imaging modality for identification and specification of the tumour. If there is any suspicion for STS, the gold standard for differentiating between STSs and benign tumours is core needle biopsy. (22, 23) Because of the complexity of the histopathologic assessment of STS, a dedicated sarcoma pathologist should review the results. Literature has demonstrated that in STS the discordance

between nonexpert pathologists and sarcoma pathologists is considerable with 9% zero agreement (one of the two pathologists did not diagnose a sarcoma) and 38% partial agreement (both pathologists diagnosed an STS but with different histopathological grade or subtype). (24)

Grading of STS

Considering the heterogeneity in presentation and outcome of STS, several grading and staging systems have been developed to classify patients in different risk groups. The 8th edition of the American Joint Committee on Cancer (AJCC) TNM staging system is a site-specific staging system that categorizes patients based on tumour size (categorized), lymph node involvement, distant metastasis and histologic grade. (25) Histological grade is defined with the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system which categorizes STS in three grades based on tumour differentiation, mitotic count, and tumour necrosis (**Table 1**).

Table 1. Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system

Tumour differentiation	
1 point	Closely resembling normal mesenchymal tissue
2 points	Histologic typing is certain
3 points	Embryonal and undifferentiated sarcoma
Mitotic count	
1 point	0-9 mitoses per 10 high-power field
2 points	10-19 mitoses per 10 high-power field
3 points	>19 mitoses per 10 high-power field
Tumour necrosis	
0 point	No necrosis
1 point	≤50% necrosis
2 points	≥50% necrosis
Histologic grade	
Grade 1	Total score of 2-3
Grade 2	Total score of 4-5
Grade 3	Total score of 6-8

The AJCC staging system aids prognostication but has some limitations as it does not include histological subtype and tumour depth, which are important independent prognostic factors regardless of histologic grade. (7, 26-29) Furthermore, it does not incorporate patient-related factors and does not provide individual prognosis. In recent years, several new prognostic tools have been developed and validated for eSTS such as Sarculator and PERSARC. (27, 28, 30-32) These tools are, in general, easier to use through applications on smartphones, more accurate as they generate individual

prognosis based on multiple characteristics that may vary simultaneously and provide more easily understood prognosis compared with the conventional TNM staging system. Furthermore, these tools have incorporated time-varying variables that consider that the prognosis varies at different time points during follow-up. (30, 32, 33)

Management of STS

Several clinical guidelines have been developed for the management of STS. (22, 23) Currently, the majority of all histological subtypes are treated uniformly. The treatment of STS occurs in a multidisciplinary team using a multimodality approach. This dedicated team consists of radiologists, pathologists, surgical and orthopaedic oncologists, and radiation and medical oncologists.

Surgery with complete surgical margins is the cornerstone in the treatment of localized eSTS. (Neo)adjuvant radiotherapy (RTX) is typically discussed in high grade eSTS with a high risk of local relapse or incomplete surgical margins. There is no clear preference concerning the timing of RTX. There is no difference in local control and overall survival after neoadjuvant or adjuvant RTX. (34-37) However, short term wound complications are less common after adjuvant RTX, while long-term morbidity such as fibrosis, oedema and joint stiffness are less common after neoadjuvant RTX due to lower radiation dose. (34-37) Considering that the short term-complications are well manageable in specialized sarcoma centres, RTX is nowadays typically offered preoperatively. (22, 38)

(Neo)adjuvant chemotherapy (CTX) may be indicated in patients with a high risk of developing distant metastases (DM) or death. Perioperative CTX is not standard of care in the management of primary eSTS. Despite multiple randomized and non-randomized studies on the role of (neo)adjuvant CTX in eSTS, the added value of CTX is still widely debated due to conflicting results. (39-50) Therefore, clinical guidelines state that perioperative CTX may be considered in selected patients on an individual basis after multidisciplinary discussion. (22)

Treatment of localized eSTS with neoadjuvant isolated limb perfusion with tumour necrosis factor-alpha (TNF-α) in combination with melphalan and (neo)adjuvant CTX with regional hyperthermia may also be considered for limb-preserving surgery after multidisciplinary discussion in specialized sarcoma centres. (22, 23, 51)

Patients with metastatic disease are usually treated in a palliative setting. In this setting the balance between life expectancy and quality of life becomes even more important. The mainstay treatment of metastatic STS is best supportive care or anthracycline-

based systemic therapy. In selected cases of isolated oligometastatic disease local treatment with surgery, RTX or interventional radiological ablation techniques may be an option. (22, 23)

Malignant peripheral nerve sheath tumour

Malignant peripheral nerve sheath tumours (MPNSTs) are a rare and aggressive subtype of STS that account for 2-6% of all STS. (5, 7, 52) MPNSTs originate from peripheral nerve supporting tissue. (1) While most STS arise *de novo* and have an unknown etiology, MPNSTs are associated with neurofibromatosis type 1 (NF1). (1) NF1 is a common, autosomal dominant disorder with a heterozygous loss-of-function germline mutation in the NF1 tumour suppressor gene. (1) This gene encodes for Neurofibromin, which is an inhibitor of RAS oncogenes. (53, 54) Activation of the RAS pathway results in cell proliferation and survival. (55)

The incidence of NF1 is estimated as 1 in 2500-3000 births. (56, 57) Patients with NF1 could develop multiple benign cutaneous and plexiform neurofibromas. These (plexiform) neurofibromas could progress into atypical neurofibromas (aNF) or atypical neurofibromatous neoplasms of uncertain biological potential (ANNUBP), (1, 58-61) which could eventually dedifferentiate to MPNSTs. However, not all precursors undergo malignant transformation. (1, 58-61)

The estimated life time risk of developing a MPNST in patients with NF1 is 8-13%. (62) Approximately 30-50% of the MPNSTs are NF1-associated. (63, 64) MPNSTs could also be sporadic or radiation-related, which account for roughly 40-60% and 5-10% of all MPNSTs, respectively. (1, 63, 64)

Historically, grading was not universally applied in MPNSTs, due to conflicting results. (65-67) However, several recent and larger studies have shown the prognostic value of the FNCLCC grading system in MPNSTs. (64, 68-71) Therefore, nowadays the FNCLCC grading system is also recommended for MPNSTs by the 2020 WHO classification of soft tissue tumours. (1) The most important source of difficulty in grading MPNSTs seems the determination of the differentiation score (**Table 1**). (71) The differentiation score for MPNSTs could be defined as:

- 1 point: resembles benign peripheral nerve sheath tumour with increased cellularity and mitotic activity.
- 2 points: overtly malignant neoplasm with characteristics of nerve sheath differentiation (i.e., marbling, and short fascicle formation).

- 3 points: overtly malignant neoplasm lacking clear morphologic features of nerve sheath differentiation, having heterologous elements or epithelioid morphology. (71)

In general, MPNSTs are treated similarly as all STS subtypes, as discussed in the previous sections.

Aim and outline of this thesis

As stated in the preceding paragraphs, STS represents a rare and heterogeneous type of cancer. This not only adds complexity to the diagnosis and treatment of STS but also presents challenges in conducting research. Despite several scientific advances in STS, including molecular diagnostics, treatment with limb sparing surgery in combination with perioperative RTX, and the development of several prediction tools, there has only been a marginal improvement in survival throughout the years. (72-75)

Over the past few decades, there has been a paradigm shift in cancer research from focusing on the homogeneity within a patient population to emphasizing the diversity or heterogeneity in presentation and clinical outcomes among patients. This concept has been commonly referred to as personalised medicine. The foundation of personalised medicine lies in delivering effective care tailored to each individual patient. In this thesis, we aimed to contribute to a more personalised and patient-tailored approach in the management of STS. We tried to achieve this goal by addressing the following three main questions:

1. **PART I:** Given the current practice,
   - what is the variation in clinical presentation and oncological outcome of patients with STS?
   - which factors influence this variation in oncological outcome?
2. **PART II:** How to better identify patients at risk and predict oncological outcome in patients with STS?
3. **PART III:** What is the current management of STS and how could prognostic tools play a role in the clinical decision making and management of STS?

The **first part** of this thesis focuses on the heterogeneity in presentation and outcome across the STS spectrum. In **chapter 2** we assessed the differences in oncological outcome in STS between the AYA (adolescents and young adults), middle-aged (40-69 years old) and elderly population (≥70 years old) and assessed whether the differences

in oncological outcome could be explained by tumour and treatment characteristics. In **chapter 3 and 4**, we aimed to identify tumour and treatment-related factors that are associated with survival and the development of DM and LR in patients with MPNST. In the final chapter of this section, **chapter 5**, we systematically reviewed the literature on immunohistochemical markers and genetic alterations in MPNSTs that are associated with survival.

The **second part** of this thesis focuses on prognostic and diagnostic research in STS. In **chapter 6** we studied whether an electronic nose could discriminate between patients with and without STS based on exhaled breath in a pilot study. In **chapter 7** we built and validated a MPNST-specific prediction tool and compared the performance of this tool with existing generic STS tools.

The **third part** concentrates on the management of STS and the application of prediction tools in the management of STS. In **chapter 8** we explored the variation in treatment recommendations and management of STS in an international survey among sarcoma experts. In **chapter 9** the role of perioperative chemotherapy in eSTS was investigated in different risk groups by stratifying patients based on the PERSARC prediction tool. In **chapter 10** we evaluated the added value of restaging for distant disease after neoadjuvant RTX in STS. **Chapter 11** provides an overview of existing prediction tools for eSTS and discusses the possible applications of prediction tools in the management of eSTS.

The final part of this thesis, the **fourth part**, contains a summary and discussion of all the chapters and outlines future perspectives for sarcoma research.

References

1. WHO Classification of Tumours: Soft Tissue and Bone Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA: a cancer journal for clinicians. 2021;71(1):7-33.
3. The Netherlands Cancer Registry. Dutch Cancer Figures (Cijfers over kanker). Netherlands Comprehensive Cancer Organisation (IKNL); 1989-2021.
4. Bleyer A, Barr R, Hayes-Lattin B, Thomas D, Ellis C, Anderson B. The distinctive biology of cancer in adolescents and young adults. Nature reviews Cancer. 2008;8(4):288-98.
5. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. European journal of cancer (Oxford, England : 1990). 2013;49(3):684-95.
6. Blay JY, Honoré C, Stoeckle E, Meeus P, Jafari M, Gouin F, et al. Surgery in reference centers improves survival of sarcoma patients: a nationwide study. Annals of oncology : official journal of the European Society for Medical Oncology. 2019;30(7):1143-53.
7. Zagars GK, Ballo MT, Pisters PW, Pollock RE, Patel SR, Benjamin RS, et al. Prognostic factors for patients with localized soft-tissue sarcoma treated with conservation surgery and radiation therapy: an analysis of 1225 patients. Cancer. 2003;97(10):2530-43.
8. Coindre JM, Terrier P, Guillou L, Le Doussal V, Collin F, Ranchère D, et al. Predictive value of grade for metastasis development in the main histologic types of adult soft tissue sarcomas: a study of 1240 patients from the French Federation of Cancer Centers Sarcoma Group. Cancer. 2001;91(10):1914-26.
9. Bedi M, King DM, Charlson J, Whitfield R, Hackbarth DA, Zambrano EV, et al. Multimodality management of metastatic patients with soft tissue sarcomas may prolong survival. Am J Clin Oncol. 2014;37(3):272-7.
10. Lochner J, Menge F, Vassos N, Hohenberger P, Kasper B. Prognosis of Patients with Metastatic Soft Tissue Sarcoma: Advances in Recent Years. Oncol Res Treat. 2020;43(11):613-9.
11. Younger E, Husson O, Bennister L, Whelan J, Wilson R, Roast A, et al. Age-related sarcoma patient experience: results from a national survey in England. BMC cancer. 2018;18(1):991.
12. Kang S, Han I, Lee SA, Cho HS, Kim HS. Unplanned excision of soft tissue sarcoma: the impact of the referring hospital. Surgical oncology. 2013;22(2):e17-22.
13. Fiore M, Casali PG, Miceli R, Mariani L, Bertulli R, Lozza L, et al. Prognostic effect of re-excision in adult soft tissue sarcoma of the extremity. Annals of surgical oncology. 2006;13(1):110-7.
14. Bourcier K, Dinart D, Le Cesne A, Honore C, Meeus P, Blay JY, et al. Outcome of Patients with Soft-Tissue Sarcomas: An Age-Specific Conditional Survival Analysis. The oncologist. 2019;24(7):e559-e64.
15. Soomers V, Husson O, Desar IME, van de Sande MAJ, de Haan JJ, Verhoef C, et al. Patient and diagnostic intervals of survivors of sarcoma: Results from the SURVSARC study. Cancer. 2020;126(24):5283-92.
16. Smolle MA, Tunn PU, Goldenitsch E, Posch F, Szkandera J, Bergovec M, et al. The Prognostic Impact of Unplanned Excisions in a Cohort of 728 Soft Tissue Sarcoma Patients: A Multicentre Study. Annals of surgical oncology. 2017;24(6):1596-605.
17. Chandrasekar CR, Wafa H, Grimer RJ, Carter SR, Tillman RM, Abudu A. The effect of an unplanned excision of a soft-tissue sarcoma on prognosis. J Bone Joint Surg Br. 2008;90(2):203-8.

18. Nakamura T, Kawai A, Hagi T, Asanuma K, Sudo A. A comparison of clinical outcomes between additional excision after unplanned and planned excisions in patients with soft-tissue sarcoma of the limb : a propensity matching cohort study. The bone & joint journal. 2021;103-b(12):1809-14.
19. Zaidi MY, Ethun CG, Liu Y, Poultsides G, Howard JH, Mogal H, et al. The impact of unplanned excisions of truncal/extremity soft tissue sarcomas: A multi-institutional propensity score analysis from the US Sarcoma Collaborative. Journal of surgical oncology. 2019;120(3):332-9.
20. Charoenlap C, Imanishi J, Tanaka T, Slavin J, Ngan SY, Chander S, et al. Outcomes of unplanned sarcoma excision: impact of residual disease. Cancer Med. 2016;5(6):980-8.
21. Liang Y, Guo TH, Xu BS, Hong DC, Qiu HB, Zhou ZW, et al. The Impact of Unplanned Excision on the Outcomes of Patients With Soft Tissue Sarcoma of the Trunk and Extremity: A Propensity Score Matching Analysis. Front Oncol. 2020;10:617590.
22. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2021;32(11):1348-65.
23. Von Mehren M, Kane JM, Agulnik M, Bui MM, Ganjoo KN, George S, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. 2018;16(5):536-63.
24. Ray-Coquard I, Montesco MC, Coindre JM, Dei Tos AP, Lurkin A, Ranchère-Vince D, et al. Sarcoma: concordance between initial diagnosis and centralized expert review in a population-based study within three European regions. Annals of oncology : official journal of the European Society for Medical Oncology. 2012;23(9):2442-9.
25. American Joint Committee on Cancer. AJCC cancer staging manual 8th ed. Chicago: Springer; 2017.
26. Kattan MW, Leung DH, Brennan MF. Postoperative nomogram for 12-year sarcoma-specific death. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(3):791-6.
27. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Development and external validation of two nomograms to predict overall survival and occurrence of distant metastases in adults after surgical resection of localised soft-tissue sarcomas of the extremities: a retrospective analysis. The Lancet Oncology. 2016;17(5):671-80.
28. van Praag VM, Rueten-Budde AJ, Jeys LM, Laitinen MK, Pollock R, Aston W, et al. A prediction model for treatment decisions in high-grade extremity soft-tissue sarcomas: Personalised sarcoma care (PERSARC). European journal of cancer (Oxford, England : 1990). 2017;83:313-23.
29. Lahat G, Tuvin D, Wei C, Anaya DA, Bekele BN, Lazar AJ, et al. New perspectives for staging and prognosis in soft tissue sarcoma. Annals of surgical oncology. 2008;15(10):2739-48.
30. Callegaro D, Miceli R, Bonvalot S, Ferguson PC, Strauss DC, van Praag VVM, et al. Development and external validation of a dynamic prognostic nomogram for primary extremity soft tissue sarcoma survivors. EClinicalMedicine. 2019;17:100215.
31. Smolle MA, Sande MV, Callegaro D, Wunder J, Hayes A, Leitner L, et al. Individualizing Follow-Up Strategies in High-Grade Soft Tissue Sarcoma with Flexible Parametric Competing Risk Regression Models. Cancers (Basel). 2019;12(1).
32. Rueten-Budde AJ, van Praag VM, van de Sande MAJ, Fiocco M. Dynamic prediction of overall survival for patients with high-grade extremity soft tissue sarcoma. Surgical oncology. 2018;27(4):695-701.

33. Rueten-Budde AJ, van Praag VM, van de Sande MAJ, Fiocco M. External validation and adaptation of a dynamic prediction model for patients with high-grade extremity soft tissue sarcoma. Journal of surgical oncology. 2021;123(4):1050-6.
34. Haas RL, Gronchi A, van de Sande MAJ, Baldini EH, Gelderblom H, Messiou C, et al. Perioperative Management of Extremity Soft Tissue Sarcomas. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(2):118-24.
35. O'Sullivan B, Davis AM, Turcotte R, Bell R, Catton C, Chabot P, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. Lancet. 2002;359(9325):2235-41.
36. O'Sullivan B, Davis A, Turcotte R, Bell R, Wunder J, Catton C, et al. Five-year results of a randomized phase III trial of pre-operative vs post-operative radiotherapy in extremity soft tissue sarcoma. Journal of Clinical Oncology. 2004;22(14_suppl):9007-.
37. Davis AM, O'Sullivan B, Turcotte R, Bell R, Catton C, Chabot P, et al. Late radiation morbidity following randomization to preoperative versus postoperative radiotherapy in extremity soft tissue sarcoma. Radiother Oncol. 2005;75(1):48-53.
38. Van Meekeren M, Fiocco M, Ho VKY, Bovée J, Gelderblom H, Haas RL. Patterns of Perioperative Treatment and Survival of Localized, Resected, Intermediate- or High-Grade Soft Tissue Sarcoma: A 2000-2017 Netherlands Cancer Registry Database Analysis. Sarcoma. 2021;2021:9976122.
39. Woll PJ, Reichardt P, Le Cesne A, Bonvalot S, Azzarelli A, Hoekstra HJ, et al. Adjuvant chemotherapy with doxorubicin, ifosfamide, and lenograstim for resected soft-tissue sarcoma (EORTC 62931): a multicentre randomised controlled trial. The Lancet Oncology. 2012;13(10):1045-54.
40. Fakhrai N, Ebm C, Kostler WJ, Jantsch M, Abdolvahab F, Dominkus M, et al. Intensified adjuvant IFADIC chemotherapy in combination with radiotherapy versus radiotherapy alone for soft tissue sarcoma: long-term follow-up of a prospective randomized feasibility trial. Wiener klinische Wochenschrift. 2010;122(21-22):614-9.
41. Frustaci S, Gherlinzoni F, De Paoli A, Bonetti M, Azzarelli A, Comandone A, et al. Adjuvant chemotherapy for adult soft tissue sarcomas of the extremities and girdles: results of the Italian randomized cooperative trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(5):1238-47.
42. Gortzak E, Azzarelli A, Buesa J, Bramwell VH, van Coevorden F, van Geel AN, et al. A randomised phase II study on neo-adjuvant chemotherapy for 'high-risk' adult soft-tissue sarcoma. European journal of cancer (Oxford, England : 1990). 2001;37(9):1096-103.
43. Petrioli R, Coratti A, Correale P, D'Aniello C, Grimaldi L, Tanzini G, et al. Adjuvant epirubicin with or without Ifosfamide for adult soft-tissue sarcoma. Am J Clin Oncol. 2002;25(5):468-73.
44. Zaidi MY, Ethun CG, Tran TB, Poultsides G, Grignol VP, Howard JH, et al. Assessing the Role of Neoadjuvant Chemotherapy in Primary High-Risk Truncal/Extremity Soft Tissue Sarcomas: An Analysis of the Multi-institutional U.S. Sarcoma Collaborative. Annals of surgical oncology. 2019;26(11):3542-9.
45. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Impact of perioperative chemotherapy and radiotherapy in patients with primary extremity soft tissue sarcoma: retrospective analysis across major histological subtypes and major reference centres. European journal of cancer (Oxford, England : 1990). 2018;105:19-27.
46. Mullen JT, Kobayashi W, Wang JJ, Harmon DC, Choy E, Hornicek FJ, et al. Long-term follow-up of patients treated with neoadjuvant chemotherapy and radiotherapy for large, extremity soft tissue sarcomas. Cancer. 2012;118(15):3758-65.

47. Pervaiz N, Colterjohn N, Farrokhyar F, Tozer R, Figueredo A, Ghert M. A systematic meta-analysis of randomized controlled trials of adjuvant chemotherapy for localized resectable soft-tissue sarcoma. Cancer. 2008;113(3):573-81.
48. Istl AC, Ruck JM, Morris CD, Levin AS, Meyer CF, Johnston FM. Call for improved design and reporting in soft tissue sarcoma studies: A systematic review and meta-analysis of chemotherapy and survival outcomes in resectable STS. Journal of surgical oncology. 2019;119(7):824-35.
49. Graham DS, van Dams R, Jackson NJ, Onyshchenko M, Eckardt MA, DiPardo BJ, et al. Chemotherapy and Survival in Patients with Primary High-Grade Extremity and Trunk Soft Tissue Sarcoma. Cancers (Basel). 2020;12(9).
50. Pasquali S, Pizzamiglio S, Touati N, Litiere S, Marreaud S, Kasper B, et al. The impact of chemotherapy on survival of patients with extremity and trunk wall soft tissue sarcoma: revisiting the results of the EORTC-STBSG 62931 randomised trial. European journal of cancer (Oxford, England : 1990). 2019;109:51-60.
51. Issels RD, Lindner LH, Verweij J, Wessalowski R, Reichardt P, Wust P, et al. Effect of Neoadjuvant Chemotherapy Plus Regional Hyperthermia on Long-term Outcomes Among Patients With Localized High-Risk Soft Tissue Sarcoma: The EORTC 62961-ESHO 95 Randomized Clinical Trial. JAMA Oncol. 2018;4(4):483-92.
52. Brennan MF, Antonescu CR, Moraco N, Singer S. Lessons learned from the study of 10,000 patients with soft tissue sarcoma. Ann Surg. 2014;260(3):416-21; discussion 21-2.
53. Kahen EJ, Brohl A, Yu D, Welch D, Cubitt CL, Lee JK, et al. Neurofibromin level directs RAS pathway signaling and mediates sensitivity to targeted agents in malignant peripheral nerve sheath tumors. Oncotarget. 2018;9(32):22571-85.
54. Brohl AS, Kahen E, Yoder SJ, Teer JK, Reed DR. The genomic landscape of malignant peripheral nerve sheath tumors: diverse drivers of Ras pathway activation. Sci Rep. 2017;7(1):14992.
55. Harrisingh MC, Perez-Nadales E, Parkinson DB, Malcolm DS, Mudge AW, Lloyd AC. The Ras/Raf/ERK signalling pathway drives Schwann cell dedifferentiation. Embo j. 2004;23(15):3061-71.
56. Uusitalo E, Leppävirta J, Koffert A, Suominen S, Vahtera J, Vahlberg T, et al. Incidence and mortality of neurofibromatosis: a total population study in Finland. J Invest Dermatol. 2015;135(3):904-6.
57. Evans DG, Howard E, Giblin C, Clancy T, Spencer H, Huson SM, et al. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. Am J Med Genet A. 2010;152a(2):327-32.
58. Beert E, Brems H, Daniëls B, De Wever I, Van Calenbergh F, Schoenaers J, et al. Atypical neurofibromas in neurofibromatosis type 1 are premalignant tumors. Genes Chromosomes Cancer. 2011;50(12):1021-32.
59. Higham CS, Dombi E, Rogiers A, Bhaumik S, Pans S, Connor SEJ, et al. The characteristics of 76 atypical neurofibromas as precursors to neurofibromatosis 1 associated malignant peripheral nerve sheath tumors. Neuro Oncol. 2018;20(6):818-25.
60. Miettinen MM, Antonescu CR, Fletcher CDM, Kim A, Lazar AJ, Quezado MM, et al. Histopathologic evaluation of atypical neurofibromatous tumors and their transformation into malignant peripheral nerve sheath tumor in patients with neurofibromatosis 1-a consensus overview. Hum Pathol. 2017;67:1-10.
61. Lemberg KM, Wang J, Pratilas CA. From Genes to -Omics: The Evolving Molecular Landscape of Malignant Peripheral Nerve Sheath Tumor. Genes (Basel). 2020;11(6).
62. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet. 2002;39(5):311-4.

63. Watson KL, Al Sannaa GA, Kivlin CM, Ingram DR, Landers SM, Roland CL, et al. Patterns of recurrence and survival in sporadic, neurofibromatosis Type 1-associated, and radiation-associated malignant peripheral nerve sheath tumors. J Neurosurg. 2017;126(1):319-29.
64. Miao R, Wang H, Jacobson A, Lietz AP, Choy E, Raskin KA, et al. Radiation-induced and neurofibromatosis-associated malignant peripheral nerve sheath tumors (MPNST) have worse outcomes than sporadic MPNST. Radiother Oncol. 2019;137:61-70.
65. Zou C, Smith KD, Liu J, Lahat G, Myers S, Wang W-L, et al. Clinical, pathological, and molecular variables predictive of malignant peripheral nerve sheath tumor outcome. Ann Surg. 2009;249(6):1014-22.
66. WHO Classification of Tumours of Soft Tissue and Bone. 4th ed: International Agency for Research on Cancer (IARC); 2013.
67. Coindre JM. Grading of soft tissue sarcomas: review and update. Arch Pathol Lab Med. 2006;130(10):1448-53.
68. Valentin T, Le Cesne A, Ray-Coquard I, Italiano A, Decanter G, Bompas E, et al. Management and prognosis of malignant peripheral nerve sheath tumors: The experience of the French Sarcoma Group (GSF-GETO). European journal of cancer (Oxford, England : 1990). 2016;56:77-84.
69. Anghileri M, Miceli R, Fiore M, Mariani L, Ferrari A, Mussi C, et al. Malignant peripheral nerve sheath tumors: prognostic factors and survival in a series of patients treated at a single institution. Cancer. 2006;107(5):1065-74.
70. Stucky C-CH, Johnson KN, Gray RJ, Pockaj BA, Ocal IT, Rose PS, et al. Malignant peripheral nerve sheath tumors (MPNST): the Mayo Clinic experience. Annals of surgical oncology. 2012;19(3):878-85.
71. Wakeman KM, Zhang QS, Bandhlish A, Cranmer LD, Ricciotti RW, Mantilla JG. Fédération Nationale Des Centres de Lutte Contre Le Cancer (FNCLCC) Grading, Margin Status and Tumor Location Associate With Survival Outcomes in Malignant Peripheral Nerve Sheath Tumors. Am J Clin Oncol. 2022;45(1):28-35.
72. Trovik C, Bauer HCF, Styring E, Sundby Hall K, Vult Von Steyern F, Eriksson S, et al. The Scandinavian Sarcoma Group Central Register: 6,000 patients after 25 years of monitoring of referral and treatment of extremity and trunk wall soft-tissue sarcoma. Acta Orthop. 2017;88(3):341-7.
73. Kollár A, Rothermundt C, Klenke F, Bode B, Baumhoer D, Arndt V, et al. Incidence, mortality, and survival trends of soft tissue and bone sarcoma in Switzerland between 1996 and 2015. Cancer Epidemiol. 2019;63:101596.
74. Sampo MM, Klintrup K, Tukiainen EJ, Böhling TO, Blomqvist CP. Improved prognosis in soft-tissue sarcoma of extremity and trunk wall. Acta Orthop. 2017;88(1):116-20.
75. Vos M, Ho VKY, Oosten AW, Verhoef C, Sleijfer S. Minimal Increase in Survival Throughout the Years in Patients with Soft Tissue Sarcoma with Synchronous Metastases: Results of a Population-Based Study. The oncologist. 2019;24(7):e526-e35.

PART I

Risk Factors and Oncological Outcome: Heterogeneity Within the Sarcoma Spectrum

Chapter 2

Age-Related Differences of Oncological Outcomes in Primary Extremity Soft Tissue Sarcoma: A Multistate Model Including 6260 Patients

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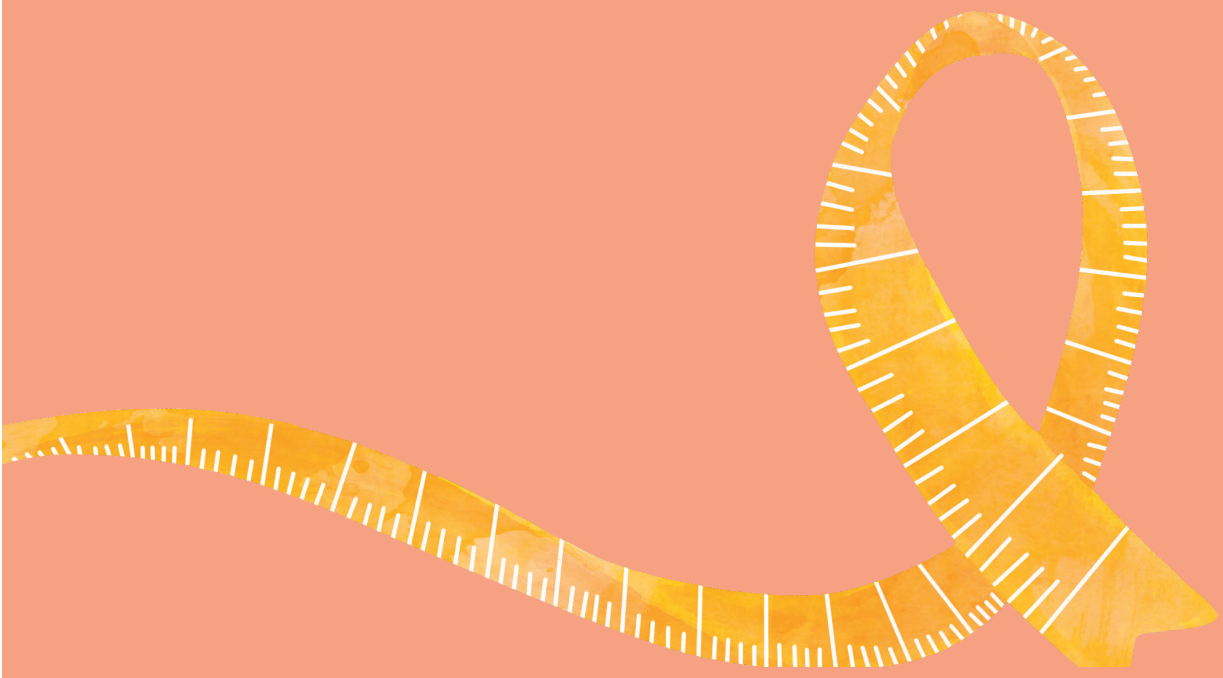

Abstract

Background

No studies extensively compared the young adults (YA, 18-39 years), middle-aged (40-69 years), and elderly (≥70 years) population with primary high-grade eSTS. This study aimed to determine whether the known effect of age on overall survival (OS) and disease progression can be explained by differences in tumour characteristics and treatment protocol among the YA, middle-aged, and elderly population in primary high-grade eSTS patients treated with curative intent.

Methods

In this retrospective multicentre study, inclusion criteria were patients with primary high-grade eSTS of 18 years and older, surgically treated with curative intent between 2000 and 2016. Cox proportional hazard models and a multistate model were used to determine the association of age on OS and disease progression.

Results

A total of 6260 patients were included in this study. YA presented more often after "whoops"-surgery or for re-resection due to residual disease, and with more deep-seated tumours. Elderly patients presented more often with grade-III and larger (≥10cm) tumours. After adjustment for the imbalance in tumour and treatment characteristics the hazard ratio for OS of the middle-aged population is 1.46 (95%CI 1.22-1.74) and 3.06 (95%CI 2.53-3.69) in the elderly population, compared with YA.

Conclusion

The effect of age on OS could only partially be explained by the imbalance in the tumour characteristics and treatment variables. The threefold higher risk of elderly could, at least partially, be explained by a higher other-cause mortality. The results might also be explained by a different tumour behaviour or suboptimal treatment in elderly compared to the younger population.

Introduction

Soft tissue sarcomas (STS) are a group of rare heterogeneous tumours of mesenchymal origin with various histologic and clinical features. The estimated incidence of STS is less than 4.7 per 100,000 persons in Northern Europe per year. (1) STSs may occur in all age groups, with a relatively high incidence in patients younger than 40 years old compared to other malignancies. (1, 2) STS represent approximately 1-2% of all adult malignancies (2, 3), and 7-8% of all malignancies in adolescents and young adults (AYA). (3, 4)

In the past, clinical trials mainly focused on the middle-aged population, in which STS is most prevalent, (3) while the AYA and elderly population remained underrepresented in these trials. (5, 6) The lack of enrolment in clinical trials of the AYA and elderly population limits our knowledge of tumours behaviour and effectiveness of STS management in these populations.

Several studies have shown relative lack of improvement in clinical outcomes in the AYA population compared to their older and younger counterparts (4, 7) and poorer disease-specific survival of the elderly patients compared to the younger counterparts. (8) With the increasing referrals for treatment of elderly patients with STS as well as the lack of improvement in the AYA population, further evaluation of factors influencing outcome for the different age groups might help in the decision-making regarding treatment strategies for the different patient groups. (4, 7, 9, 10)

Therefore, the primary aim of this study is to evaluate differences in overall survival (OS) and disease progression among age groups of patients with a primary high-grade eSTS treated with a curative intent. The secondary aim is to determine whether potential differences in outcome can be explained by differences in tumour and treatment characteristics among the different age groups.

Methods

Study design and population

This is a retrospective multicentre study of surgically treated primary high-grade eSTS patients. Local institutional ethics board approval was obtained prior to the study. Patients were identified from 21 participating specialized sarcoma centres or registries (**Appendix A**).

All patients with primary high-grade (FNCLCC II/III) eSTS of 18 years and older that were surgically treated with curative intent between 2000-2016 with correctly

registered time-to-events were included. Patients undergoing re-excision after unplanned sarcoma excision were also included. Exclusion criteria were:

- presentation with local recurrence (LR) or distant metastases (DM)
- intermediate malignancy tumours, Kaposi, and paediatric sarcomas
- patients receiving (neo)adjuvant treatment other than radiotherapy (RTX) or chemotherapy (CTX) (e.g., isolated limb perfusion)
- patients who died or were censored at the day of definitive surgery
- patients of whom age or time-to-event data was missing.

Variables

Patient information, tumour characteristics, treatment-related variables and survival data were obtained from medical records or sarcoma registries. Age was determined as age at time of surgery. Patients were categorized into three age groups (YA: 18-39, middle-aged: 40-69, elderly: 70+). Size was measured as the maximum diameter of tumour mass on imaging-techniques or based on pathological report. The FNCLCC grading-system was used for tumour grading. A tumour partially or entirely deep to the investing fascia was classified as deep. Histological subtypes were retrieved from pathology reports and were classified into 7 categories according to the World Health Organization classification (11): leiomyosarcoma (LMS), liposarcoma (LPS), myxofibrosarcoma (MF), undifferentiated pleomorphic sarcoma and (pleomorphic) STS not-otherwise-specified (UPS/NOS), malignant peripheral nerve sheath tumour (MPNST), synovial sarcoma (SS) and other. The "other"-category included angiosarcoma, adult rhabdomyosarcoma and other histological subtypes under-presented in our data. A "whoops"-surgery was defined as a surgical procedure in which the mass was assumed to be benign but final pathologic diagnosis after surgery showed an STS. Surgical margin was classified as R0 (negative, defined as no ink on tumour) or R1-2 (microscopically/macroscopically positive). No central pathology review for the diagnosis and surgical margin was performed in this study. Due to the retrospective and multicentre nature of this study, it was not possible to centrally review 6260 eSTS cases. Since only expert centres were included in this study, we believe central review would not significantly improve the paper to warrant such an effort. All centres generally adhered to the ESMO-guidelines for diagnosis, treatment, and follow-up. (12)

LR was defined as the first radiological evidence of malignant recurrence at or near the primary tumour bed. DM was defined as the first radiological or pathological evidence of recurrence at any other side outside the primary tumour bed. For the date of LR and DM, the date of tissue biopsy was used if the diagnosis was pathologically confirmed, otherwise the date of radiological examination was used.

Endpoints of the study were OS, LR and DM.

Statistical analysis

All statistical analyses were performed in the statistical program R (version 3.6.3). (13) Patient demographics and baseline characteristics were described with proportions for categorical variables and means with standard deviations (SD) or medians with interquartile ranges (IQR). Differences in categorical variables were tested with the Chi-square test or Fisher's exact test. Bonferroni-correction for differences in tumour and treatment variables between the age groups was used to account for multiple testing.

OS was defined as the time interval between definitive surgery and date of death or date of last follow-up. Time-to-LR and time-to-DM was defined as the time interval between definitive surgery and date of LR or DM respectively, or date of last follow-up. Median follow-up was computed with the reversed Kaplan-Meier estimator. Kaplan-Meier plots for OS and cumulative incidence of LR (CILR) and DM (CIDM) plots were constructed to compare the YA, middle-aged and elderly age groups. The CILR and CIDM were estimated using competing risk analyses, with death as competing event. Differences in time-to-event outcomes were evaluated with the log-rank test or the Peto-Wilcoxon test if the proportional hazard (PH) assumption was violated. Missing values were imputed for the Cox PH models using multiple imputation (m=20). Pooled estimates were computed using Rubin's rules.

A multistate model was built to assess the association between age and disease progression. A multistate model is an extension of competing risk analyses, in which transitions to and from intermediate events are modelled. (14) **Figure 1** depicts the multistate model used in this study. Every patient starts in the initial state after definitive surgery, alive with no evidence of disease (ANED). A patient stays in this state until disease progression, death, or censoring. If a patient first develops a LR and afterwards a DM, the patient will move from ANED to LR to DM. If a patient first develops a DM and afterwards a LR, the patient will move from ANED to DM and remains in DM. If a patient is diagnosed with a LR and DM simultaneously (synchronous relapse) the patient will move directly to the DM-state.

Multivariable Cox PH models were used to estimate the effect of age on OS and for each transition. The models were adjusted for tumour and treatment characteristics. The tumour characteristics were histology, grade, size, depth, and tumour site. The treatment characteristics were surgical margin, radiotherapy, and chemotherapy. We assessed the PH-assumption visually using the Schoenfeld-residuals. We used state

occupancy plots to visualize the probability of being in a state at different time point after surgery for the three age groups.

P-values ≤0.05 were considered statistically significant. Results from the Cox PH models were described in hazard ratios (HRs) with corresponding 95% confidence intervals (CI). All statistical tests were two-sided. The packages "mstate", "mcprsk" and "survival" were used for the multistate model and survival analyses, and the package "mice" was used for multiple imputations.

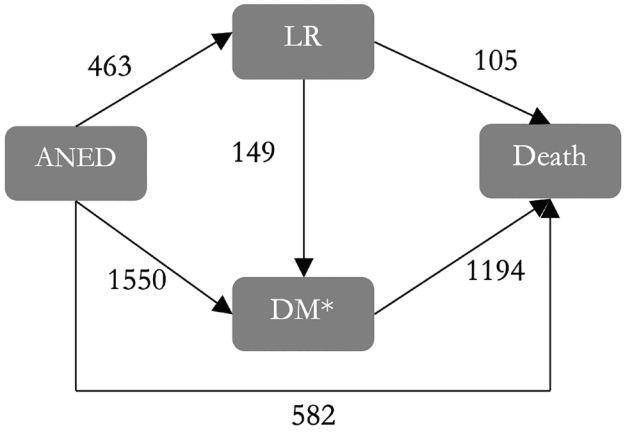

Figure 1. Disease progression of eSTS in a multistate model along with number of patients moving from one state to another

The states are indicated by blocks and the transitions are indicated by arrows.

*Patients with synchronous relapse (LR+DM) move to the DM-state. If a patient first develops a DM and afterwards a LR, the patient will remain in the DM-state.

Results

Patient population

A total of 6268 patients were eligible for this study. Two patients due to missing age, three patients due to missing time-to-event data and three patients without follow-up were excluded, resulting in 6260 patients that were included (**Figure 2**). The ages ranged between 18-100 years (median, IQR: 63, 49-74). The population was categorized into three age groups: the YA (n=841, 13.4%), the middle-aged (n=3217; 51.4%) and the elderly population (n=2202; 35.2%) (**Table 1**). The female:male ratio in the total population was 1: 1.24. The median follow-up time was 49.4 months (95%CI 47.1–52.3).

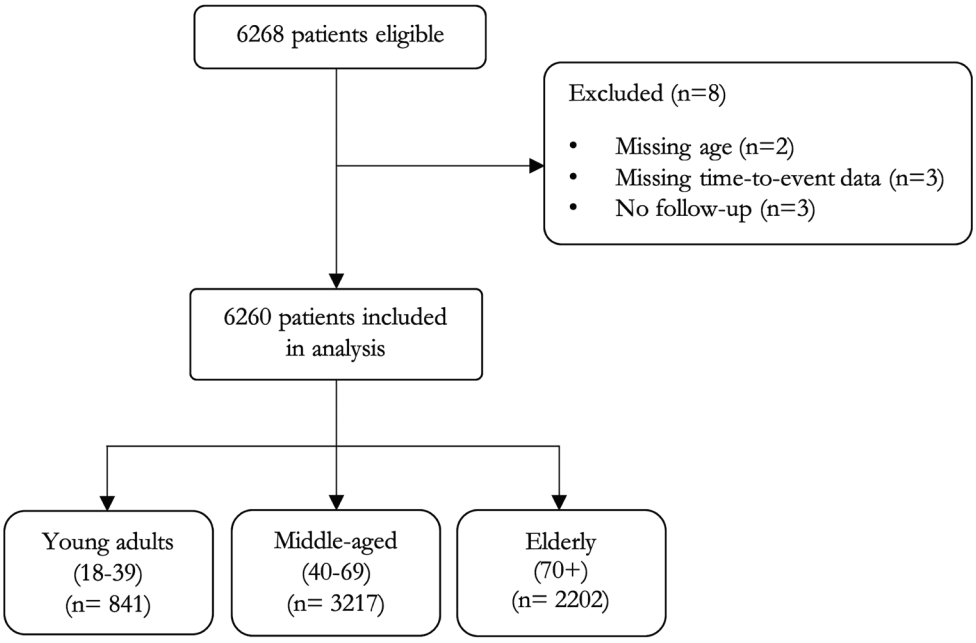

Figure 2. Flow diagram for patients included in the study
N: number of patients

Differences in tumour characteristics

YA presented more often after "whoops"-surgery or for re-resection due to residual disease compared with both the middle-aged and elderly population. Also, YA had significantly more deep-seated tumours compared with the middle-aged, and elderly population, while elderly presented more often with grade-III and large (≥10cm) tumours compared with the YA and middle-aged population.

SS, MPNST and LPS were significantly more often diagnosed in YA compared with the middle-aged and elderly population, whereas UPS and NOS were diagnosed more often in elderly compared with the YA and middle-aged population. LMS and MF were more frequent in the middle-aged and elderly population compared with YA. No significant difference was found between the middle-aged and elderly population for LMS and MF (**Table 1**). **Figure 3** describes the age distribution for the main histologic subtypes.

Table 1. Tumour and treatment characteristics

	All patients (n= 6260)	YA (n= 841)	Middle-aged (n= 3217)	Elderly (n= 2202)	p-value*
Gender					
Male	3466 (55.4)	464 (55.2)	1815 (56.4)	1187 (53.9)	
Female	2793 (44.6)	377 (44.8)	1401 (43.6)	1015 (46.1)	0.182
Missing	*1*	*0*	*1*	0	
Histology					
LMS	657 (10.5)	50 (5.95)	336 (10.5)	271 (12.3)	
LPS	1002 (16.0)	191 (22.7)	569 (17.7)	242 (11.0)	
MF	1095 (17.5)	42 (4.99)	599 (18.6)	454 (20.6)	
UPS and NOS	1948 (31.1)	96 (11.4)	959 (29.8)	893 (40.6)	
MPNST	353 (5.64)	98 (11.7)	186 (5.79)	69 (3.14)	
SS	570 (9.11)	267 (31.7)	254 (7.90)	49 (2.22)	
Other	631 (10.1)	97 (11.5)	312 (9.70)	222 (10.1)	<0.001
Missing	*4*	*0*	*2*	*2*	
Grade					
2	1008 (24.6)	169 (29.2)	585 (27.3)	254 (18.4)	
3	3096 (75.4)	410 (70.8)	1560 (72.7)	1126 (81.6)	<0.001
high grade (not further specified)	2156	262	1072	822	
Size					
< 5 cm	1510 (24.9)	239 (29.7)	802 (25.8)	469 (21.9)	
5-10 cm	2383 (39.3)	323 (40.2)	1199 (38.5)	861 (40.2)	
≥10 cm	2165 (35.7)	242 (30.1)	1112 (35.7)	811 (37.9)	<0.001
Missing	*202*	*37*	*104*	*61*	
Depth					
Deep	4095 (70.1)	601 (76.3)	2126 (71.0)	1368 (66.6)	
Superficial	1744 (29.9)	187 (23.7)	870 (29.0)	687 (33.4)	<0.001
Missing	*421*	*53*	*221*	*147*	
Site					
Lower extremity	4750 (75.9)	647 (76.9)	2501 (77.8)	1602 (72.8)	
Upper extremity	1509 (24.1)	194 (23.1)	715 (22.2)	600 (27.2)	<0.001
Missing	*1*	*0*	*1*	*0*	
Presentation					
Primary	3814 (78.8)	489 (73.2)	1928 (78.1)	1397 (82.0)	
Whoops/residue	1028 (21.2)	179 (26.8)	542 (21.9)	307 (18.0)	<0.001
Missing	*1418*	*173*	*747*	*498*	
Type of surgery					
Limb sparing	5059 (93.9)	674 (95.1)	2590 (93.9)	1795 (93.4)	
Amputation	330 (6.12)	35 (4.94)	169 (6.13)	126 (6.56)	0.306
Missing	*871*	*132*	*458*	*281*	

	All patients (n= 6260)	YA (n= 841)	Middle-aged (n= 3217)	Elderly (n= 2202)	p-value*
Resection margin					
R0	5338 (87.9)	737 (89.8)	2769 (89.2)	1832 (85.4)	
R1-R2	732 (12.1)	84 (10.2)	336 (10.8)	312 (14.6)	<0.001
Missing	*190*	*20*	*112*	*58*	
Radiotherapy					
No	3016 (48.2)	379 (45.1)	1460 (45.4)	1177 (53.5)	
Yes	3239 (51.8)	461 (54.9)	1,753 (54.6)	1025 (46.5)	<0.001
Missing	*5*	*1*	*4*	*0*	
Chemotherapy					
No	5240 (83.7)	593 (70.5)	2526 (78.5)	2121 (96.3)	
Yes	1019 (16.3)	248 (29.5)	690 (21.5)	81 (3.68)	<0.001
Missing	*1*	*0*	*1*	*0*	
Radiotherapy (detailed)					
No radiotherapy	3017 (48.6)	379 (45.4)	1459 (45.8)	1179 (53.8)	
Adjuvant	2033 (32.7)	262 (31.4)	1062 (33.4)	709 (32.4)	
Neoadjuvant	1135 (18.3)	190 (22.8)	647 (20.3)	298 (13.6)	
Neo- and adjuvant	24 (0.387)	4 (0.479)	16 (0.503)	4 (0.183)	<0.001
Missing	*51*	*6*	*33*	*12*	
Chemotherapy (detailed)					
No chemotherapy	5241 (84.1)	593 (70.8)	2529 (79.1)	2119 (96.4)	
Adjuvant	560 (8.98)	109 (13.0)	394 (12.3)	57 (2.59)	
Neoadjuvant	190 (3.05)	64 (7.65)	119 (3.72)	7 (0.318)	
Neo- and adjuvant	243 (3.90)	71 (8.48)	156 (4.88)	16 (0.728)	<0.001
Missing	*26*	*4*	*19*	*3*	

N: number of patients, YA: young adults, LMS: Leiomyosarcoma, LPS: Liposarcoma, MF: Myxofibrosarcoma, UPS: Undifferentiated pleomorphic sarcoma, NOS: Sarcoma, not otherwise specified, MPNST: Malignant peripheral nerve sheath tumour, SS: synovial sarcoma

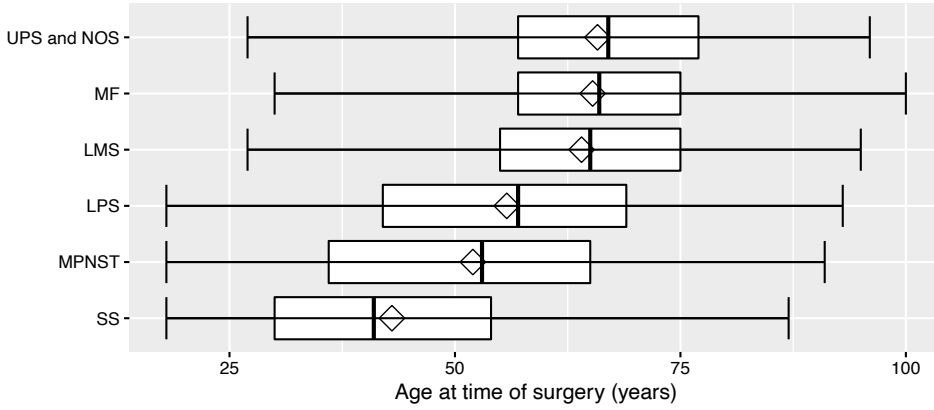

Figure 3. Age distribution for histologic subtypes

Boxes represent the 25th 50th and 75th quartiles, end of horizontal bars represent 1.5 times the inter-quartile range. Rhombus represents the mean.

LMS: Leiomyosarcoma, LPS: Liposarcoma, MF: Myxofibrosarcoma, UPS: Undifferentiated pleomorphic sarcoma, NOS: Sarcoma, not otherwise specified, MPNST: Malignant peripheral nerve sheath tumour, SS: synovial sarcoma

Differences in treatment

Elderly had significantly more R1-R2 resections compared with the YA and middle-aged population. RTX and CTX were more often offered in the YA and middle-aged population compared with elderly. Also, there was a significant difference in CTX use between the YA and middle-aged population.

Differences in outcome

There was a significant difference among the age groups for all oncological outcomes (**Figure 4**). The 5-year OS in the YA, middle-aged and elderly population, is 78.4% (95%CI 75.0-81.9), 70.3% (95%CI 68.4-72.3) and 50.0% (95%CI 47.3-52.9) respectively (**Table 2**).

Age was significantly associated with OS in the univariate model (**Figure 4a**). After adjustment for the presentation and treatment variables the association between age and OS decreased but remained significant (HR middle-aged: 1.46 (95%CI 1.22-1.74), HR elderly: 3.06 (95%CI 2.53-3.69), YA as reference) (**Table 3**).

Age demonstrated a significant effect on the cause-specific hazard of LR (**Figure 4b**). The difference in the cause-specific hazard of LR between the YA and middle-aged population could entirely be explained by the imbalance in tumour and treatment characteristics (HR middle-aged: 1.38 (95%CI 0.978-1.95), YA as reference). Difference in the cause-specific hazard of LR between the YA and elderly population

could partially be explained by the imbalance in tumour and treatment characteristics (HR elderly: 2.20 (95%CI 1.53-3.16), YA as reference) (**Table 3**, **Transition 1**). Also, age demonstrated a significant effect on the cause-specific hazard of DM (**Figure 4c**). The imbalance in tumour and treatment characteristics does not seem to explain the difference in the cause-specific hazard of DM among the age groups (HR middle-aged: 1.26 (95%CI 1.07-1.49), HR elderly: 1.23 (95%CI 1.02-1.48), YA as reference) (**Table 3, Transition 2**). HRs for the elderly were the highest for transition 3 (ANED à Death) and 5 (LR à Death) (**Table 3**). Cumulative incidence plots for LR and DM stratified by age group and histology are depicted in **Appendix C**.

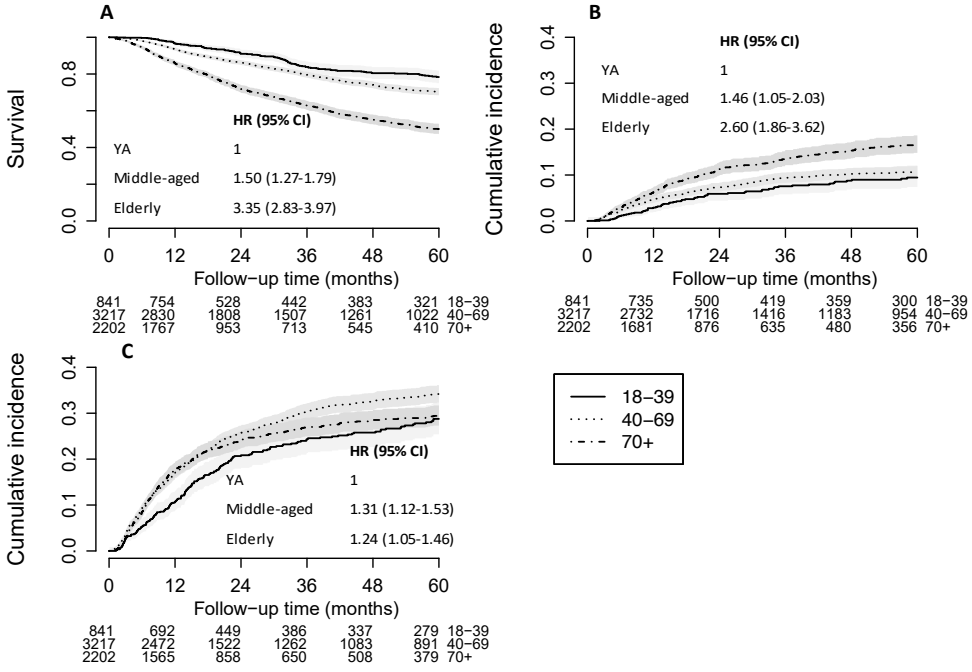

Figure 4. Kaplan Meier curves

A. Overall survival (log-rank: p <0.001)

B. Cumulative incidence of local recurrence (log-rank: p <0.001)

C. Cumulative incidence of distant metastasis (Peto-Wilcoxon: p = 0.001)

YA: Young adults, HR: Hazard ratio, CI: confidence interval

Table 2. Oncological outcome stratified by age group

Oncological outcome	Young adults (95% CI)	Middle-aged (95% CI)	Elderly (95% CI)
Overall survival			
2 years	91.1% (89.1-93.3)	86.2% (84.9-87.5)	71.8% (69.8-74.0)
5 years	78.4% (75.0-81.9)	70.3% (68.4-72.3)	50.0% (47.3-52.9)
10 years	66.7% (61.5-72.3)	58.4% (55.6-61.2)	23.7% (20.3-27.7)
Cumulative incidence of LR			
1 year	2.91% (1.76-4.05)	4.67% (3.94-5.41)	6.33% (5.30-7.35)
2 years	5.90% (4.19-7.61)	7.34% (6.39-8.30)	11.2% (9.79-12.6)
5 years	9.45% (7.14-11.8)	10.7% (9.46-11.9)	16.6% (14.7-18.5)
Cumulative incidence of DM			
1 year	10.8% (8.64-12.9)	17.0% (15.7-18.3)	17.6% (16.0-19.2)
2 years	20.8% (17.9-23.8)	25.6% (24.0-27.2)	24.1% (22.2-26.0)
5 years	28.8% (25.2-32.3)	34.2% (32.3-36.1)	29.4% (27.2-31.6)
Overall survival after first LR			
1 year	79.8% (69.8-91.3)	66.7% (61.3-72.6)	59.9% (54.0-66.4)
2 years	54.0% (41.6-70.0)	49.1% (43.2-55.9)	45.5% (39.4-52.5)
5 years	41.5% (29.3-58.8)	32.0% (25.9-39.5)	22.7% (17.2-29.8)
Overall survival after first DM			
1 year	70.1% (63.9-76.9)	59.6% (56.4-63.0)	35.9% (31.8-40.4)
2 years	42.4% (35.7-50.4)	37.1% (33.8-40.7)	15.8% (12.6-19.8)
5 years	21.7% (15.9-29.6)	16.8% (14.0-20.1)	6.28% (4.19-9.42)

CI: confidence interval, LR: Local recurrence, DM: Distant metastasis

State occupancy probabilities

The probability of occupying the LR-state is similar for each age group over time. The probability of occupying the DM-state in the first year after definitive surgery is the highest in elderly patients compared with the YA and middle-aged population. The probability of occupying the DM decreases after a year because of people moving to the Death-state (**Figure 5**).

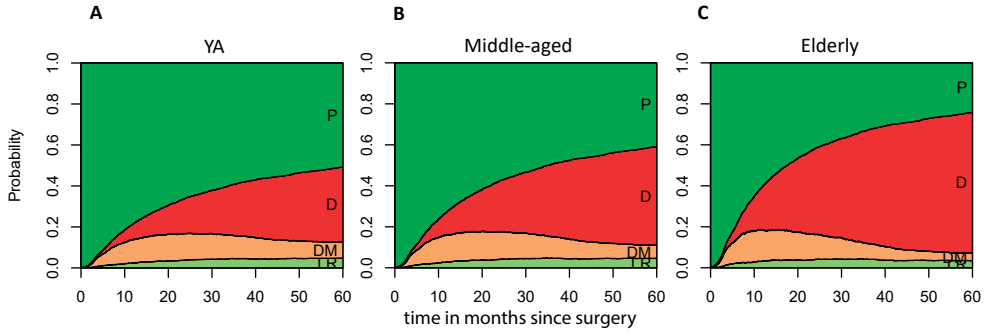

Figure 5. State occupation probabilities for three patients with the same profile in each age group

Panel A: patient in the YA group with a grade III, deep-seated, MPNST of 10 cm of the lower limb treated with RT and R0-resection. Panel B: patient in the middle-aged group with the same patient profile as A. Panel C: patient in the elderly group with the same patient profile as A.

The distance between two curves denotes the probability of being in a specific state at a specific time after surgery.

YA: Young Adults, P: Alive no evidence of disease, D: Death, DM: Distant metastasis, LR: Local recurrence

Table 3. Hazard ratios of age for overall survival and all transitions in the multistate model

	OS	**TRANS 1 ANED →LR**	**TRANS 2 ANED →DM**	**TRANS 3 ANED →Death**	**TRANS 4 LR →DM**	**TRANS 5 LR →Death**	**TRANS 6 DM →Death**
Age	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
YA	1	1	1	1[$]	1	1	1
Middle-aged	1.46 (1.22-1.74)	1.38 (0.978-1.95)	1.26 (1.07-1.49)		1.26 (0.703-2.25)	1.40 (0.506-3.89)	1.26 (1.03-1.54)
Elderly	3.06 (2.53-3.69)	2.20 (1.53-3.16)	1.23 (1.02-1.48)	5.93 (4.85-7.25)	0.792 (0.419-1.49)	4.54 (1.66-12.4)	2.20 (1.76-2.74)

Adjusted for histology, grade, size, depth and tumour site, surgical margin, (neo)adjuvant radiotherapy and (neo)adjuvant chemotherapy. For transition 3 (ANED→Death), we grouped the YA and middle-aged population due to the relatively small number of patients in this transition for these age groups. For transition 5 (LR→Death), we only adjusted for tumour characteristics due to the relatively small number of patients in this transition. Appendix B includes the full multistate model including het HRs of the adjusted variables.

[$] For transition 3 the YA and Middle-aged group were combined in one group.

OS: Overall survival, ANED: Alive, no evidence of disease, LR: Local recurrence, DM: Distant metastasis, HR: Hazard ratio, YA: Young adults

Discussion

This study showed significant differences among the YA, middle-aged and elderly population in tumour characteristics, treatment strategies and all oncological outcomes. The differences in OS among the age groups could partially be explained by the imbalance in tumour and treatment characteristics. The difference in LR rates between the YA and middle-aged could entirely be explained by the imbalance in these baseline characteristics, but the difference between the YA and elderly population could only partially be explained by the imbalance. Differences in DM rates among the age groups seem not to be explained by the imbalance in tumour and treatment characteristics among the groups.

It is noteworthy that YA presented more often after "whoops"-surgery. This is in line with the findings of Young et al. which showed that AYA were more vulnerable to incorrect diagnosis compared with the elderly population. (15) This could be explained by the overall lower prevalence of malignant tumours in YA which makes medical professionals less aware that STS can also affect YA. Another explanation for the higher "whoops" rates in the YA compared with the elderly is that YA presented with smaller tumours, which might mistakenly be considered benign more frequently.

This study showed a higher overall mortality in the elderly population compared with their younger counterparts, which is in accordance with previous studies. (8, 16) Also, elderly have a more than six- and five-times higher risk of dying in the ANED and LR state, respectively. Since OS was taken as an endpoint rather than disease specific survival, this was to be expected since elderly obviously have a higher risk of dying of natural causes. However, other studies have also shown an increased sarcoma-specific mortality in the older population. (8, 9, 16, 17)

The elderly presented with larger (≥10cm) and more grade-III tumours compared with the YA and middle-aged population. Also, the variation in histological subtypes in the elderly was different than in the younger populations. Elderly were more frequently diagnosed with UPS and NOS, which tend to be more aggressive tumours. (18) All these tumour characteristics could partly explain the impaired OS in the elderly.

Also, elderly had more positive resection margins. This might be due the fact that elderly presented more often with unresectable tumours, or that surgeons chose to perform less extensive resections to improve quality of life in the elderly. Also, elderly patients are less often offered radiation or chemotherapy, probably due to pre-existing comorbidities and reduced physical and psychological reserves. (9, 10, 19)

The lower rates of RTX use in the elderly might explain the higher LR rates in this age group, as this study showed a HR of 0.56 for the transition from ANED à LR in those who received RTX. Also, RTX was associated with an improvement in OS (HR: 0.85). CTX was not associated with an improvement in OS, but was associated with the transition from ANED à DM (HR: 1.3). This could probably be explained by confounding by indication, as patients with higher risk of developing a DM are more likely to receive CTX.

After adjustment for the imbalance in tumour and treatment variables, the association between age and OS decreases, suggesting that worse OS in the elderly may only partially be explained by the imbalance of tumour and treatment variables. However, it has been suggested that elderly have a more aggressive tumour biology and a weaker tumour-specific immune response, (20, 21) which might be another explanation for decreased survival. This is supported by the finding that the probability of developing DM in the first year after surgery is higher for the elderly compared with the younger counterparts with the same tumour and treatment characteristics. Besides elderly have a higher risk of developing a DM, they also have a higher risk of dying after DM. The 1-year OS after first DM was 35.9% in the elderly compared with 59.6% in the middle-aged population. We did not have any information about the treatment regimens after disease progression, but a potential explanation for the declined OS in elderly could also be a less aggressive treatment approach in this population.

This study found an increased risk of LR in the elderly population compared with YA, in accordance with previous reports, (8, 22) Also, an increased but less evident risk of DM was found in the middle-aged and elderly population compared with YA. After adjustment for tumour and treatment characteristics, the difference in cause-specific hazard of LR among the age groups decreased. However, the association for the cause-specific hazard of DM remained the same after adjustment, suggesting that the imbalance in measured tumour and treatment characteristics does not explain the difference in DM rate. These findings are in line with a previous report of Biau et al., which showed that the effect of age on DM could hardly be explained by presentation and treatment variables. (22) Yet, unmeasured, or not-fully modelled explanatory confounders could also, at least partially, explain the remaining association. However, our study included more than twice as many patients compared with Biau et al. which made it possible to adjust for more variables without overfitting the models. (22)

Limitations and strengths

This study has several limitations due to its retrospective design. First, missing data and patients lost to follow-up were present in our dataset, probably resulting in selection bias due to selective lost to follow-up. We have used multiple imputations

to reduce the bias. Furthermore, the association among the age groups and clinical outcome could be explained by other variables we did not include in our analysis, such as treatment characteristics of progressive disease, resulting in residual confounding. Also, we combined patients with R1 and R2 resections in one group, as more detailed information about surgical margins was not available in all centres. Finally, we were unable to assess the disease-specific survival which would provide more insight into the influence of tumour and treatment characteristics on the effect of age. Nevertheless, to our knowledge this is the largest multicentre study to date examining age-related differences in oncological outcome for primary high-grade eSTS patients surgically treated with curative intent.

Conclusion

In this large multicentre study, we have observed a significant decrease in OS and increase in LR and DM rate with increasing age. This can only partially be explained by differences in tumour and treatment characteristics, suggesting that eSTS may have a more aggressive tumour behaviour in elderly patients when compared with their younger counterparts, which may coincide with a weaker tumour-specific immune response in elderly patients.

References

1. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. European journal of cancer (Oxford, England : 1990). 2013;49(3):684-95.
2. Lahat G, Lazar A, Lev D. Sarcoma epidemiology and etiology: potential environmental and genetic factors. The Surgical clinics of North America. 2008;88(3):451-81, v.
3. Ferrari A, Sultan I, Huang TT, Rodriguez-Galindo C, Shehadeh A, Meazza C, et al. Soft tissue sarcoma across the age spectrum: a population-based study from the Surveillance Epidemiology and End Results database. Pediatric blood & cancer. 2011;57(6):943-9.
4. Bleyer A, Barr R, Hayes-Lattin B, Thomas D, Ellis C, Anderson B. The distinctive biology of cancer in adolescents and young adults. Nature reviews Cancer. 2008;8(4):288-98.
5. Townsley CA, Selby R, Siu LL. Systematic review of barriers to the recruitment of older patients with cancer onto clinical trials. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(13):3112-24.
6. Shaw PH, Hayes-Lattin B, Johnson R, Bleyer A. Improving enrollment in clinical trials for adolescents with cancer. Pediatrics. 2014;133 Suppl 3:S109-13.
7. Keegan TH, Ries LA, Barr RD, Geiger AM, Dahlke DV, Pollock BH, et al. Comparison of cancer survival trends in the United States of adolescents and young adults with those in children and older adults. Cancer. 2016;122(7):1009-16.
8. Imanishi J, Chan LWM, Broadhead ML, Pang G, Ngan SY, Slavin J, et al. Clinical Features of High-Grade Extremity and Trunk Sarcomas in Patients Aged 80 Years and Older: Why Are Outcomes Inferior? Frontiers in surgery. 2019;6:29.
9. Lahat G, Dhuka AR, Lahat S, Lazar AJ, Lewis VO, Lin PP, et al. Complete soft tissue sarcoma resection is a viable treatment option for select elderly patients. Annals of surgical oncology. 2009;16(9):2579-86.
10. Boden RA, Clark MA, Neuhaus SJ, A'Hern J R, Thomas JM, Hayes AJ. Surgical management of soft tissue sarcoma in patients over 80 years. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2006;32(10):1154-8.
11. WHO Classification of Tumours: Soft Tissue and Bone Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020.
12. Leyvraz S, Jelic S. ESMO Minimum Clinical Recommendations for diagnosis, treatment and follow-up of soft tissue sarcomas. Annals of oncology : official journal of the European Society for Medical Oncology. 2005;16 Suppl 1:i69-70.
13. R Development Core Team. R: A language and environment for statistical computing. 3.6.3 ed. Vienna, Austria: R Foundation for Statistical Computing; 2010.
14. Aalen OO, Johansen S. An Empirical Transition Matrix for Non-Homogeneous Markov Chains Based on Censored Observations. Scandinavian Journal of Statistics. 1978;5(3):141-50.
15. Younger E, Husson O, Bennister L, Whelan J, Wilson R, Roast A, et al. Age-related sarcoma patient experience: results from a national survey in England. BMC cancer. 2018;18(1):991.
16. Hoven-Gondrie ML, Bastiaannet E, Ho VK, van Leeuwen BL, Liefers GJ, Hoekstra HJ, et al. Worse Survival in Elderly Patients with Extremity Soft-Tissue Sarcoma. Annals of surgical oncology. 2016;23(8):2577-85.
17. Al-Refaie WB, Habermann EB, Dudeja V, Vickers SM, Tuttle TM, Jensen EH, et al. Extremity soft tissue sarcoma care in the elderly: insights into the generalizability of NCI Cancer Trials. Annals of surgical oncology. 2010;17(7):1732-8.

18. Penel N, Coindre JM, Giraud A, Terrier P, Ranchere-Vince D, Collin F, et al. Presentation and outcome of frequent and rare sarcoma histologic subtypes: A study of 10,262 patients with localized visceral/soft tissue sarcoma managed in reference centers. Cancer. 2018;124(6):1179-87.
19. Samet J, Hunt WC, Key C, Humble CG, Goodwin JS. Choice of cancer therapy varies with age of patient. Jama. 1986;255(24):3385-90.
20. Marusyk A, DeGregori J. Declining cellular fitness with age promotes cancer initiation by selecting for adaptive oncogenic mutations. Biochimica et biophysica acta. 2008;1785(1):1-11.
21. Flood PM, Urban JL, Kripke ML, Schreiber H. Loss of tumor-specific and idiotype-specific immunity with age. The Journal of experimental medicine. 1981;154(2):275-90.
22. Biau DJ, Ferguson PC, Turcotte RE, Chung P, Isler MH, Riad S, et al. Adverse effect of older age on the recurrence of soft tissue sarcoma of the extremities and trunk. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011;29(30):4029-35.

Appendix

Appendix A. Included centres

The included centres were Aarhus University Hospital (Aarhus, Denmark), Netherlands Cancer Institute (Amsterdam, the Netherlands), Haukeland University Hospital (Bergen, Norway), Universitatsmedizin (Berlin, Germany), Royal Orthopeadic Hospital (Birmingham, UK), Sahlgrenska University Hospital (Gothenberg, Sweden), Medical University Graz (Graz, Austria), University Medical Centre Groningen (Groningen, the Netherlands), Nationwide cancer registry for bone and soft tissue tumours (Japan), Leiden University Medical Centre (Leiden, the Netherlands), Linköping University Hospital (Linköping, Sweden), The Royal Marsden (London and Surrey, UK), Skåne University Hospital (Lund, Sweden), Radboud Medical Centre (Nijmegen, the Netherlands), The Norwegian Radium Hospital (Oslo, Norway), Erasmus Medical Centre (Rotterdam, the Netherlands), Royal Netional Orthopeadic Hospital (Stanmore, UK), Karolinska University Hospital (Stockholm, Sweden), Mount Sinai Hospital (Toronto, Canada) and Umeå University Hospital (Umeå, Sweden).

Appendix B

Table 1. Hazard ratios of age for overall survival and all transitions in the multivariable multistate model, including the hazard ratios of adjusted variables

	OS	TRANS 1 ANED →LR	TRANS 2 ANED →DM	TRANS 3 ANED →Death	TRANS 4 LR →DM	TRANS 5 LR → Death	TRANS 6 DM → Death
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Age group							
YA	1	1	1	1[$]	1	1	1
Middle-aged	1.46 (1.22-1.74)	1.38 (0.978-1.95)	1.26 (1.07-1.49)		1.26 (0.703-2.25)	1.40 (0.506-3.89)	1.26 (1.03-1.54)
Elderly	3.06 (2.53-3.69)	2.20 (1.53-3.16)	1.23 (1.02-1.48)	5.93 (4.85-7.25)	0.792 (0.419-1.49)	4.54 (1.66-12.4)	2.20 (1.76-2.74)
Grade							
II	1	1	1	1	1	1	1
III	1.91 (1.65-2.21)	1.14 (0.874-1.50)	1.59 (1.36-1.86)	1.87 (1.43-2.44)	1.45 (0.917-2.30)	1.92 (1.08-3.41)	1.39 (1.15-1.69)
Margin							
R0	1	1	1	1	1	-	1
R1-R2	1.30 (1.14-1.48)	3.12 (2.52-3.87)	1.06 (0.910-1.23)	1.30 (1.01-1.66)	0.953 (0.657-1.38)	-	1.29 (1.10-1.52)
Histology							
LMS	1	1	1	1	1	1	1
LPS	0.542 (0.439-0.671)	0.848 (0.531-1.36)	0.410 (0.332-0.505)	0.711 (0.480-1.05)	0.386 (0.161-0.925)	0.648 (0.157-2.68)	1.04 (0.805-1.35)
MF	0.790 (0.659-0.947)	1.67 (1.12-2.50)	0.531 (0.438-0.644)	1.12 (0.807-1.55)	0.292 (0.136-0.627)	0.818 (0.242-2.77)	1.02 (0.812-1.28)
UPS and NOS	0.902 (0.765-1.06)	1.55 (1.05-2.27)	0.611 (0.514-0.725)	1.26 (0.937-1.70)	0.442 (0.214-0.913)	0.835 (0.252-2.77)	1.21 (0.989-1.49)
MPNST	1.27 (1.02-1.59)	2.36 (1.46-3.82)	0.766 (0.602-0.975)	1.43 (0.930-2.21)	0.646 (0.279-1.50)	1.30 (0.323-5.23)	1.92 (1.47-2.51)
Other	1.04 (0.856-1.27)	2.17 (1.42-3.32)	0.668 (0.541-0.825)	1.46 (1.02-2.09)	0.268 (0.114-0.629)	1.51 (0.445-5.16)	1.30 (1.02-1.66)
SS	0.963 (0.767-1.21)	1.21 (0.718-2.03)	0.691 (0.551-0.866)	0.827 (0.509-1.34)	0.586 (0.232-1.48)	1.48 (0.336-6.52)	1.30 (0.996-1.69)
Size (cm)							
per 5 units increase	1.41 (1.36-1.46)	1.24 (1.14-1.35)	1.38 (1.33-1.44)	1.35 (1.26-1.45)	1.09 (0.942-1.27)	1.22 (1.03-1.43)	1.14 (1.09-1.21)

	OS	TRANS 1 ANED →LR	TRANS 2 ANED →DM	TRANS 3 ANED →Death	TRANS 4 LR →DM	TRANS 5 LR → Death	TRANS 6 DM → Death
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Depth							
Superficial	1	1	1	1	1	1	1
Deep	1.37 (1.21-1.55)	1.11 (0.882-1.40)	1.45 (1.26-1.68)	1.18 (0.956-1.45)	1.60 (1.03-2.48)	1.15 (0.724-1.83)	1.14 (0.968-1.34)
Extremity							
Lower	1	1	1	1	1	1	1
Upper	1.09 (0.976-1.21)	1.21 (0.982-1.49)	0.987 (0.870-1.12)	1.22 (1.01-1.48)	0.719 (0.486-1.06)	0.907 (0.584-1.41)	0.981 (0.850-1.13)
Radiotherapy							
No	1	1	1	1	1	-	1
Yes	0.847 (0.766-0.936)	0.560 (0.458-0.686)	1.28 (1.14-1.43)	0.539 (0.451-0.643)	1.66 (1.15-2.39)	-	0.921 (0.808-1.05)
Chemotherapy							
No	1	1	1	1	1	-	1
Yes	0.946 (0.815-1.10)	1.06 (0.780-1.43)	1.28 (1.12-1.46)	1.03 (0.742-1.44)	0.740 (0.448-1.22)	-	0.733 (0.620-0.865)

OS: Overall survival, LR: Local recurrence, DM: Distant metastasis, HR: Hazard ratio, YA: Young Adults, LMS: Leiomyosarcoma, LPS: Liposarcoma, MF: Myxofibrosarcoma, UPS: Undifferentiated pleomorphic sarcoma, NOS: Sarcoma, not otherwise specified, MPNST: Malignant peripheral nerve sheath tumour, SS: synoviosarcoma

[$] For transition 3 the AYA and Middle-aged group were combined into one group

Appendix C

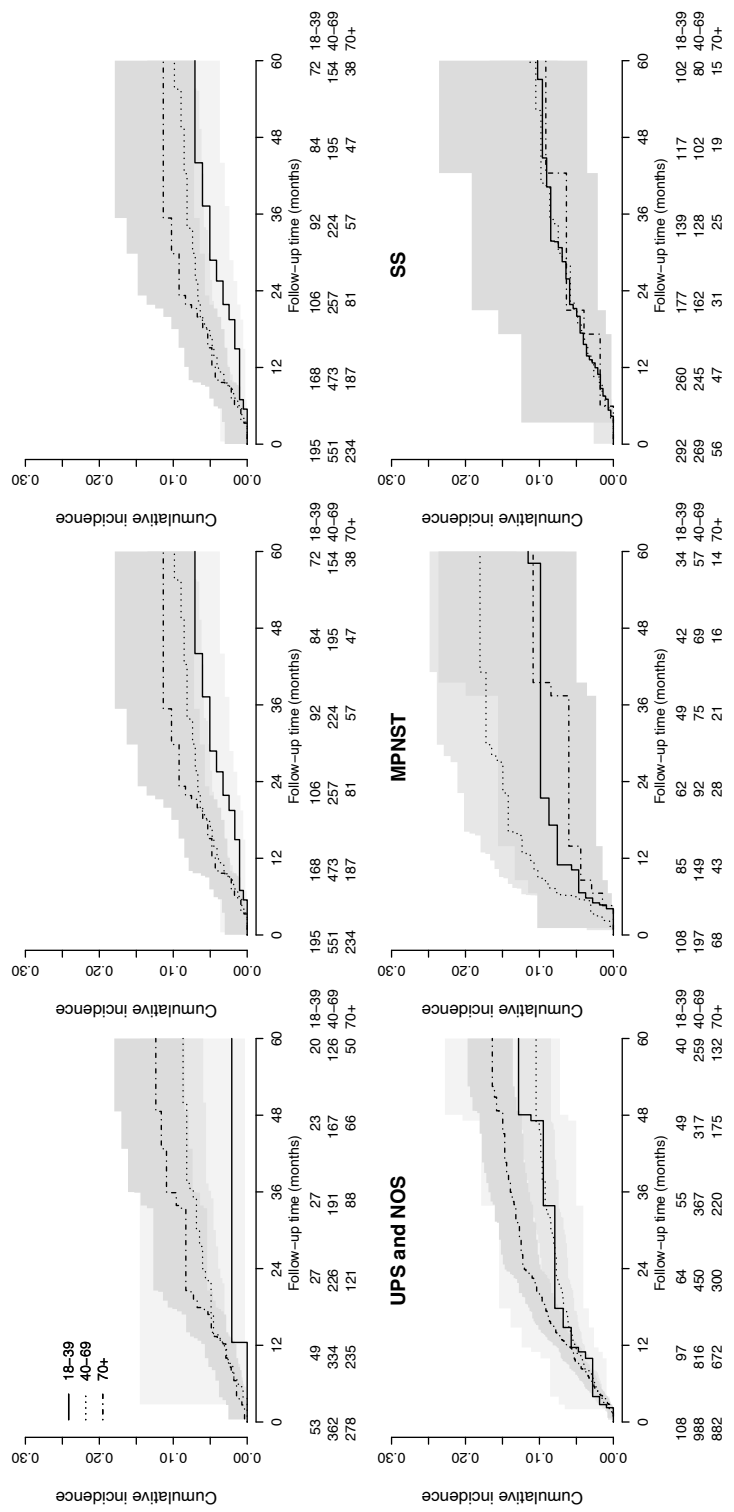

Figure 1. Cumulative Incidence of Local Recurrence

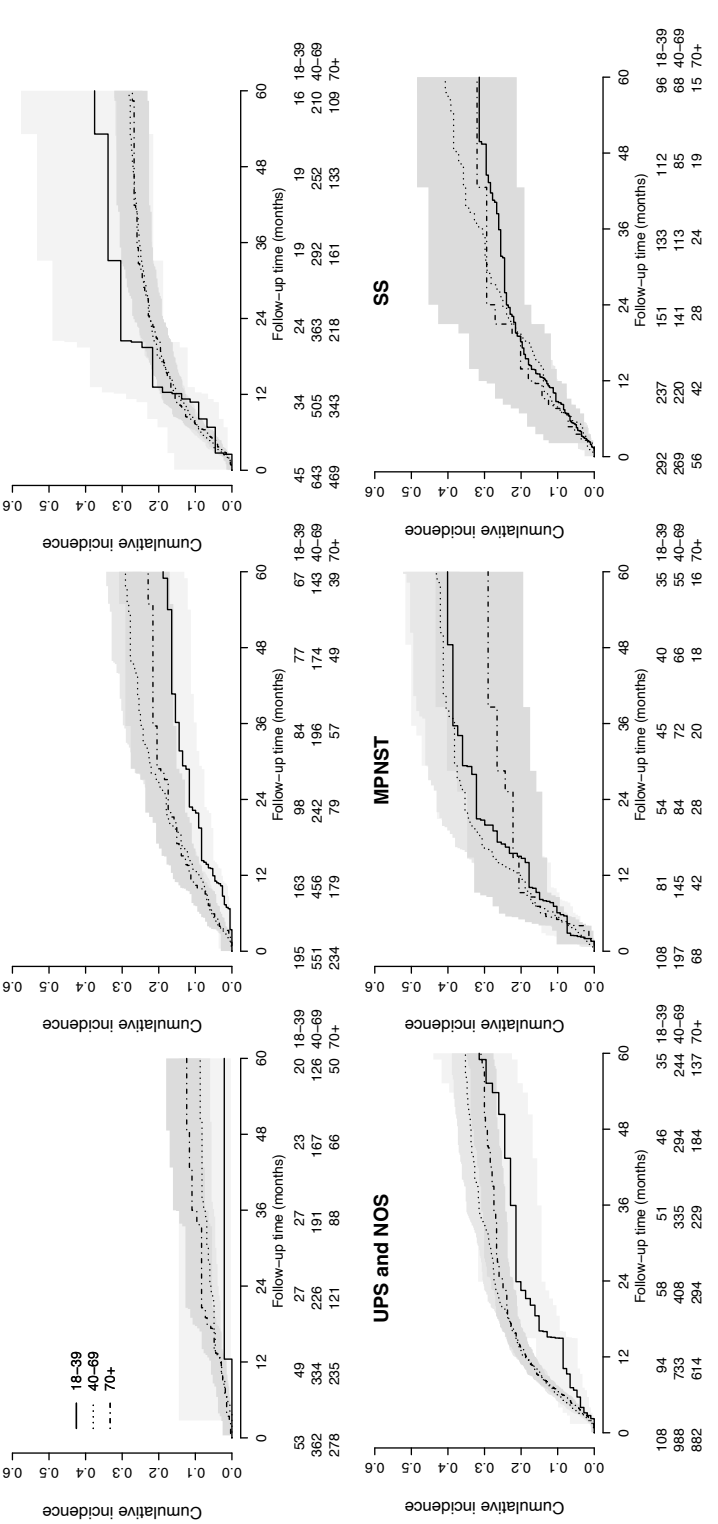

Figure 2. Cumulative Incidence of Distant Metastasis

Chapter 3

The Association of Metastasis Pattern and Management of Metastatic Disease with Oncological Outcomes in Patients with Malignant Peripheral Nerve Sheath Tumours: A Multicentre Cohort Study

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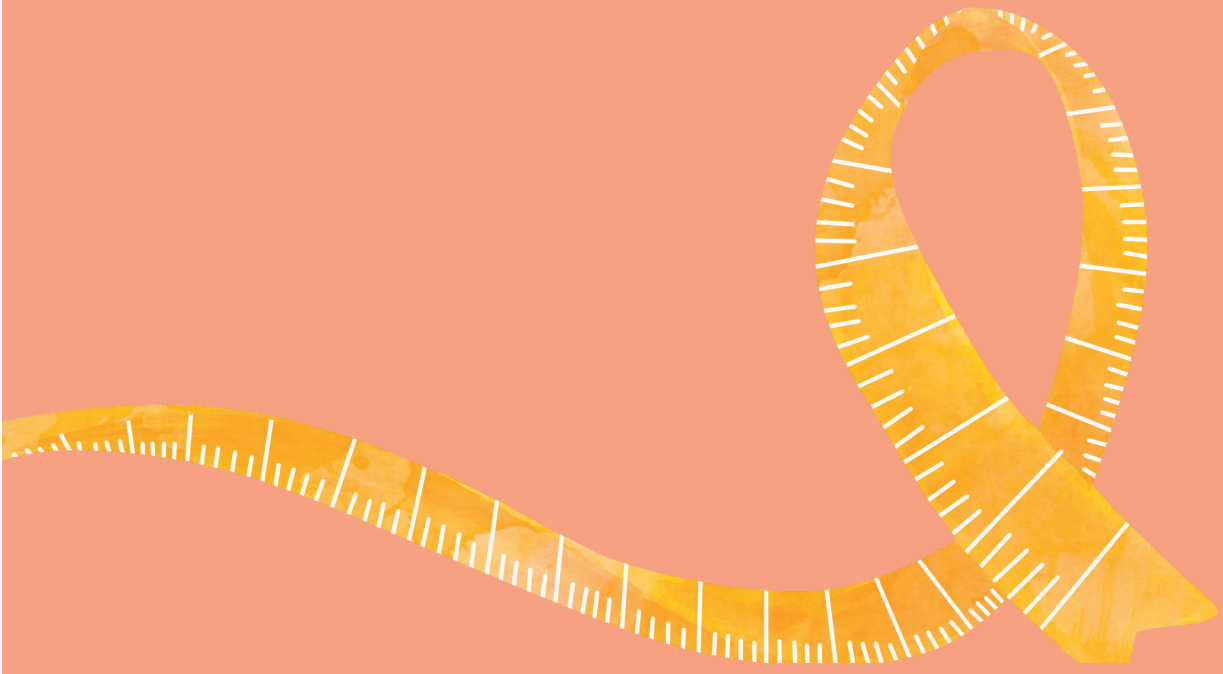
Cancers (Basel). 2021 Oct 12;13(20):5115.



Abstract

Background

This multicentre cohort study aimed to identify clinicopathologic and treatment-related factors associated with the development of distant metastasis (DM) and with overall survival (OS) after DM in patients with malignant peripheral nerve sheath tumours (MPNST).

Methods

All patients diagnosed with primary MPNST from 1988 to 2019 who were surgically treated for the primary tumour were included. Multivariable Cox regression analyses were performed to identify factors associated with DM and OS after DM.

Results

A total of 383 patients were included in this analysis, of which 150 developed metastatic disease. No differences in clinicopathologic characteristics and clinical outcome were found between patients with synchronous and metachronous DM. neurofibromatosis type 1 (NF1), high grade, tumour size, triton and R2 resections were independent risk factors for the development of DM. NF1, and more than two metastasis sites were independently associated with worse OS after DM. Metastasectomy, chemotherapy and the metastatic site category 'other' were associated with prolonged survival after DM.

Conclusion

This analysis provides important insights into clinicopathologic and treatment factors associated with outcomes in metastatic MPNST. Besides that, NF1-status is associated with a higher risk of DM, it is also independently associated with worse survival in metastatic MPNST.

Introduction

Approximately 30% of the patients with primary high-grade soft tissue sarcoma (STS) face metastatic disease within five years after primary treatment. (1-3) STS metastasize mainly to the lungs. (4, 5) The median survival after distant metastasis (DM) is 1-2 years. (4, 6, 7) Metastatic disease is usually treated in a palliative setting. The mainstay treatment of metastatic STS is systemic therapy and metastasectomy for metachronous lung metastasis if the disease-free interval ≥1 year. (8) Especially in this setting, the right balance between life expectancy and quality of life is important.

A better understanding of factors associated with metastatic disease and survival of metastatic disease may help to find a better balance between quantity and quality of life and enhance clinical decision-making. Several studies have assessed prognostic factors in metastatic STS. (5, 6, 9-14) However, studies on prognostic factors in metastatic malignant peripheral nerve sheath tumours (MPNSTs), a specific subtype of STS, are limited.

In contrast to other STS subtypes, MPNSTs can originate within a (plexiform) neurofibroma, can occur in patients with neurofibromatosis type 1 (NF1), and can present with partial rhabdomyoblastic differentiation (triton tumour). (15, 16) In addition, the conventional three levels grading system, the FNCLCC grade, cannot be applied to MPNSTs due to its poor prognostic value. (17)

Identification of MPNST patients more likely to develop DM and accurate prognosis after DM may guide clinical decision-making and result in a better balance between quantity and quality of life. Therefore, we sought to characterize the impact of clinicopathologic and treatment characteristics on clinical outcomes in patients with metastatic MPNST treated in 9 sarcoma centres in The Netherlands.

Methods

Patient population

A retrospective cohort study of the 9 Dutch sarcoma centres, the MONACO study, was undertaken after approval of the institutional review boards of the participating centres. All patients diagnosed with pathologically proven primary MPNST from 1988 to 2019 who were surgically treated for the primary tumour were included in this study. Patients with uncertain pathological reports or uncertain diagnosis based on available information during follow-up were excluded. In addition, patients with incorrectly registered time-to-event outcomes and patients who presented with local recurrence who were previously resected elsewhere were excluded.

Variables

Patient, tumour and treatment characteristics and survival data were obtained from medical records. Age was determined as age at the time of diagnosis. The American Society of Anaesthesiologist (ASA) classification system was used to categorize patients' physical status. (18) Size was measured as the maximum diameter of tumour mass on imaging or based on pathology report. Tumour grade was categorized as low and high grade based on the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system. A tumour originating from below the investing fascia was categorized as deep seated. A tumour was categorized as NF1-associated by confirmed genetic testing of a NF1 mutation or by clinical evaluation. (19) Surgical margin was categorized as R0 (microscopically negative), R1 (microscopically positive) or R2 (macroscopically positive). Tumour site was categorized as extremity, central (thorax, abdomen, pelvis, retroperitoneal), and head and neck. Triton status was extracted from pathological reports and was concluded either when stated as such in the report or when MPNST with rhabdomyoblastic differentiation was reported. Radiotherapy-associated MPNST was defined as previously delivered radiotherapy on the same site as the primary tumour bed. Metastatic sites were based on radiological reports. Metastatic site was categorized as pulmonary, extra-pulmonary with or without pulmonary metastasis, and other. Extra-pulmonary metastases were defined as liver, bone, brain, and peritoneal metastasis. The other category included lymph node metastasis and other rare metastatic sites. Number of metastatic sites was categorized as one site vs two or more sites. The disease-free interval (DFI) was defined as the time between definitive surgery and the development of the first distant metastasis (DM) and was categorized as synchronous, ≤1 year and, >1 year after definitive surgery.

DM was defined as the first radiological or pathological evidence of recurrence at any other site outside the primary tumour bed. DM at presentation (synchronous metastasis) was defined as DM diagnosed within 3 months after date of diagnosis. DM developed after 3 months was categorized as metachronous metastasis.

Endpoints of this study were DM and OS.

Statistical analysis

All statistical analyses were performed in R (version 4.1.0). (20) Baseline characteristics were described with proportions for categorical variables and means with standard deviations or medians with interquartile ranges (IQRs) for continuous variables.

Overall survival (OS) was defined as the time interval between definitive surgery and death or date of last follow-up. Time-to-DM was defined as the time interval between definitive surgery and date of fist DM. Median follow-up was estimated

with the reversed Kaplan-Meier estimator. Cumulative incidence of DM (CIDM) was estimated with death as competing event. Differences in time-to-event outcomes were evaluated with the log-rank test.

Multivariable Cox Proportional Hazards (PH) models were used to estimate the effect of several covariates on the development of metachronous DM and on OS after first DM. The model for the development of DM included age, NF1, grade, tumour size, presence of triton, depth, tumour site, radiotherapy (RTX) for primary tumour, chemotherapy (CTX) for primary tumour, and surgical margin. The model assessing the effect of different covariates on OS after first DM included age, NF1, size of primary tumour, grade, presence of triton, depth, number of metastatic sites, site of metastasis, DFI, metastasectomy, and CTX for metastatic disease.

Proportional hazards were assessed visually with the Schoenfeld residuals.

Missing values were imputed using multiple imputations (MI) (m=20) and estimates were pooled using Rubin's rule. (21)

A p-value ≤0.05 was considered statistically significant. Results from the Cox PH models were described in hazard ratios (HR) with 95% confidence intervals (CI). All statistical tests were two-sided. The packages 'mice' for MI, 'survival', 'rms', and 'survminer' were used for the survival and competing risk analyses.

Results

A total of 481 patients were included in the MONACO study. Patients who presented with a local recurrence (n = 6), who were not treated surgically for the primary tumour (n = 64), and patients with incomplete time-to-event information (n = 28) were excluded in this analysis (**Appendix Figure 1**). Of the 383 patients included in this study (**Appendix Table 1**), 150 developed a DM during follow-up. The median follow-up was 47.9 months. The median follow-up in patients with metastatic MPNST was 23.7 months. Patient and tumour characteristics are summarized in **Table 1**. Thirty-six patients had a distant metastasis at presentation (9.40%). Fifty-seven patients (38.0%) had an MPNST in association with NF1. The median number of outpatient clinic visits of the total cohort after initial treatment was 6 times (IQR 3-6) in the first year, 3 times (IQR 3-4) in the second year, and 3 times (IQR 2-3) in the 4th and 5th year.

Table 1. Baseline characteristics of 150 metastatic MPNST patients

Variable	Overall (*n* = 150)	2-Year Survival after DM diagnosis (95% CI)
Age (years)		
Median (IQR)	44 (29–59)	
Gender		
Female	69 (46.0%)	23.1 (14.9–35.8)
Male	81 (54.0%)	24.6 (16.7–36.3)
ASA		
I	70 (55.1%)	26.4 (17.8–39.1)
II	50 (39.4%)	19.9 (11.2–35.2)
III	7 (5.51%)	21.4 (4.20–100)
Missing	*23*	
Tumour size (mm)		
Median (IQR)	70 (40–113)	
Missing	*14*	
Depth		
Superficial	17 (12.1%)	45.8 (26.9–77.7)
Deep	124 (87.9%)	22.0 (15.8–30.9)
Missing	*9*	
Grade		
Low grade	8 (5.4%)	37.5 (15.3–91.7)
High grade	141 (94.6%)	22.5 (16.5–30.8)
Missing	*1*	
Site		
Extremities	70 (46.7%)	27.3 (18.5–40.3)
Central	70 (46.7%)	21.2 (13.4–33.6)
Head and neck	*10 (6.7%)*	20.0 (5.79–69.1)
NF1		
No	91 (61.5%)	33.1 (24.5–44.6)
Yes	57 (38.5%)	10.5 (4.94–22.4)
Missing	*2*	
Neurofibroma		
Not in neurofibroma	130 (87.8%)	25.3 (18.8–34.2)
Within neurofibroma	18 (12.2%)	11.1 (3.01–41.0)
Missing	*2*	
Triton		
No	133 (89.9%)	23.8 (17.6–32.3)
Yes	15 (10.1%)	19.6 (5.82–65.7)
Missing	*2*	
RTX-associated		

Variable	Overall (*n* = 150)	2-Year Survival after DM diagnosis (95% CI)
No	140 (94.0%)	25.0 (18.6–33.4)
Yes	9 (6.0%)	11.1 (1.75–70.5)
Missing	*1*	
Site of metastasis		
Pulmonary only	89 (59.7%)	11.8 (4.83–29.1)
Extrapulmonary (± lung)	38 (25.5%)	24.6 (17.0–35.6)
Other	22 (14.8%)	38.1 (22.1–65.7)
Missing	*1*	
Number of metastatic sites		
1 site	120 (80.5%)	25.8 (18.9–35.1)
2 or more sites	29 (19.5%)	13.8 (5.55–34.3)
Missing	*1*	
Metastasectomy		
No	99 (71.7%)	14.3 (8.82–23.3)
Yes	39 (28.3%)	57.1 (43.2–75.6)
Missing	*12*	
Chemotherapy		
No	80 (58.0%)	31.1 (22.3–43.4)
Yes	58 (42.0%)	19.5 (11.5–33.1)
Missing	*12*	

N: number of patients, DM: Distant metastasis, CI: Confidence interval, IQR: Interquartile range, ASA: American Society of Anaesthesiologist classification, NF1: neurofibromatosis type 1, RTX: radiotherapy

Most of the patients with synchronous metastases had a metastasis at one site (80.6%). Also, the majority of the patients with a first or second metachronous metastasis had the metastasis at one site (82.0% and 80.0%, respectively). Most metastases were located in the lung (66.7%, 75.6% and 63.3%, respectively) (**Table 2**). Synchronous metastases and first metachronous metastases were mainly treated with chemotherapy (53.3% and 37.6%, respectively) or surgery (30.0% and 28.2%, respectively) (**Table 3**). Most patients with second metachronous metastasis did not receive any treatment (33.3%). Doxorubicin monotherapy was the mostly delivered first-line chemotherapy.

Table 2. Metastasis pattern in MPNST

Variable	Metastasis at Diagnosis (*n* = 36)	First Metachronous Metastasis (*n* = 123)	Second Metachronous Metastasis (*n* = 30)
Number of different metastasis sites			
1	29 (80.6%)	100 (82.0%)	23 (80.0%)
2	5 (13.9%)	18 (14.8%)	5 (13.3%)
≥3	2 (5.56%)	4 (3.28%)	2 (6.67%)
Missing	*0*	*3*	*0*
Site			
Lung	24 (66.7%)	93 (75.6%)	19 (63.3%)
Liver	5 (13.9%)	9 (7.32%)	3 (10.0%)
Lymph node	5 (13.9%)	8 (6.50%)	5 (16.7%)
Bone	3 (8.33%)	17 (13.8%)	4 (13.3%)
Brain	1 (2.78%)	2 (1.63%)	2 (6.67%)
Peritoneal	5 (13.9%)	5 (4.07%)	2 (6.67%)
Other	3 (8.33%)	14 (11.4%)	4 (13.3%)
Missing	*0*	*1*	*0*

N: number of patients

Table 3. Treatment pattern in metastatic MPNST

Variable	Metastasis at Diagnosis (*n* = 36)	First Metachronous Metastasis (*n* = 123)	Second Metachronous Metastasis (*n* = 30)
Treatment of metastasis			
No treatment	5 (16.7%)	31 (26.5%)	10 (33.3%)
Metastasectomy	7 (23.3%)	26 (23.1%)	6 (20.0%)
Metastasectomy + RTX	-	4 (3.42%)	-
Metastasectomy + CTX	1 (3.33%)	1 (0.86%)	1 (3.33%)
Metastasectomy + RTX + CTX	1 (3.33%)	1 (0.86%)	2 (6.67%)
RTX	2 (6.67%)	11 (9.40%)	6 (20.0%)
CTX	12 (40.0%)	35 (29.9%)	4 (13.3%)
RTX + CTX	1 (3.33%)	7 (5.98%)	1 (3.33%)
RFA + CTX	1 (3.33%)	-	-
Missing	6	8	0
Treatment modality for metastasis			
No treatment	5 (16.7%)	31 (26.5%)	10 (33.3%)
Metastasectomy	9 (30.0%)	33 (28.2%)	9 (30.0%)
RTX	4 (13.3%)	23 (19.7%)	9 (30.0%)
CTX	16 (53.3%)	44 (37.6%)	8 (26.7%)
RFA	1 (3.33%)	-	-
Missing	*6*	*8*	*0*
First-line chemotherapy regimen			
Doxorubicin monotherapy	8 (50.0%)	13 (35.1%)	4 (50.0%)
Epirubicin monotherapy	1 (6.25%)	2 (5.41%)	-

Variable	Metastasis at Diagnosis (*n* = 36)	First Metachronous Metastasis (*n* = 123)	Second Metachronous Metastasis (*n* = 30)
Ifosfamide monotherapy	-	5 (13.5%)	1 (12.5%)
Doxorubicin + ifosfamide	3 (18.8%)	7 (18.9%)	2 (25.0%)
Epirubicin + ifosfamide	-	1 (2.70%)	-
Other	4 (25.0%)	9 (24.3%)	1 (12.5%)
Missing	*0*	*7*	*0*

N: number of patients, RTX: Radiotherapy, CTX: Chemotherapy, RFA: Radiofrequency ablation

Differences in synchronous and first metachronous metastases

The incidence of synchronous DM was 9.40%. The incidence of metachronous DM was 30.5% at 5 years. As patients may develop both a synchronous and metachronous DM, the 5-year cumulative risk of a DM is 37.6%. MPNST patients with synchronous and first metachronous metastases were similar in respect to their baseline characteristics (**Appendix Table 2**). The median survival of patients with synchronous metastasis was 11.5 months (95% CI 8.11-19.3) compared with 8.28 months (95% CI 7.33-9.89) in patients with first metachronous metastasis (**Figure 1**). Patients diagnosed with a DM within 1 year and after 1 year after primary treatment had a median survival of 7.43 months (95% CI 4.90-9.50) and 9.89 (95% CI 7.95-19.8), respectively.

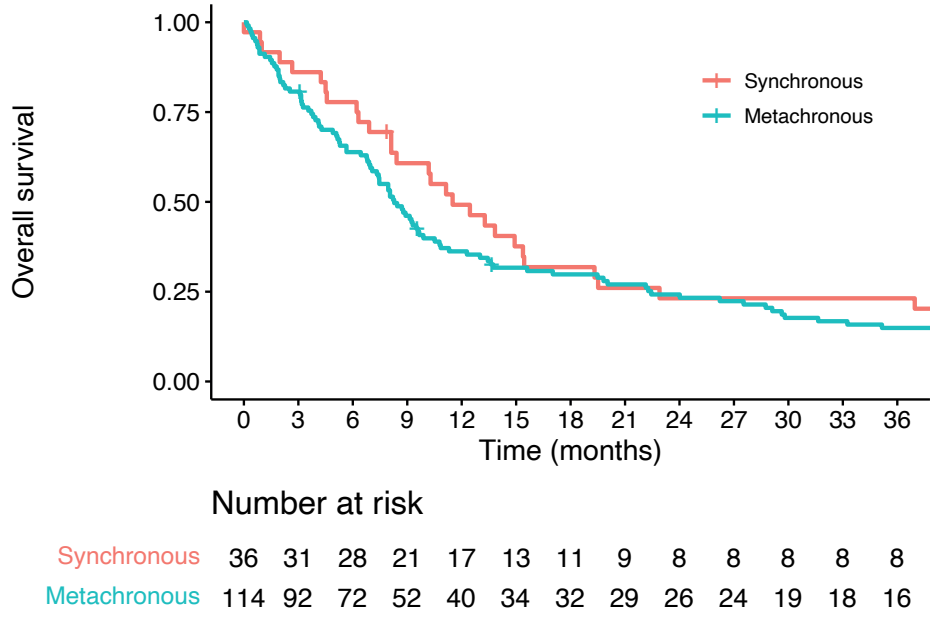


Figure 1. Survival plot of patients with synchronous vs. metachronous metastasis

p-value: Computed with log-rank test. Number at risk: Number of patients at risk of experiencing an event (death) at each time point (months) for synchronous and metachronous metastasis.

Risk factors for the development of metachronous metastatic disease in primary MPNST

Patients with NF1 associated MPNST had a higher risk of developing DM. The 2-year CIDM in NF1 patients was 35.9% compared with 18.1% in no-NF1 patients (univariable HR 1.70; 95% CI 1.18-2.45) (**Figure 2A**). The increased risk of DM could only partially be explained by the imbalance in tumour and treatment characteristics in the multivariable cause-specific Cox model (HR 1.50; 95% CI 1.00-2.24) (**Figure 3**). Also, high grade, tumour size, triton, and R2 resections were independently associated with the development of DM.

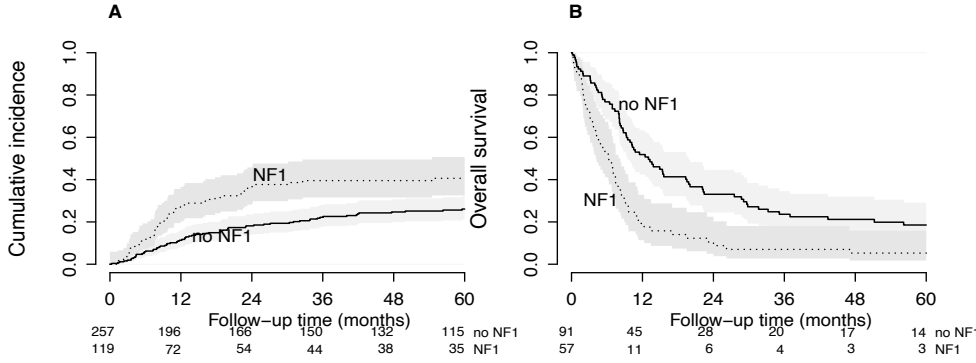


Figure 2A. Cumulative incidence of distant metastasis and **B.** overall survival after distant metastasis stratified by neurofibromatosis-1 status

Risk factors for overall survival in metastatic MNPST

The median OS after metastatic MPNST was 8.9 months, with a 2-year OS of 23.9%. Patients with NF1-associated MNPST had a worse 2-year OS (10.5%) compared with no-NF1 patients (33.1%) (median OS: 6.31 and 13.0 months, respectively) (**Table 1**). The increased risk of mortality after DM in NF1 patients could not be explained by the imbalance of other tumour and treatment characteristics (HR 2.56; 95% CI 1.68-3.90) (**Figure 4**). Number of metastasis sites were also independently associated with a worse OS after DM. The metastatic site category 'other', metastasectomy and chemotherapy for metastatic disease were independently associated with prolonged OS. **Figure 2B** depicts the overall survival of MPNST after the development of DM stratified by NF1.

Covariates	HR	95%CI
Age (per 10 years)	1.03	(0.926 – 1.14)
NF1		
No	1	
Yes	1.50	(1.00 – 2.25)
Grade		
Low grade	1	
High grade	2.59	(1.23 – 5.47)
Tumor size (per 5 cm)	1.24	(1.06 – 1.46)
Triton		
No	1	
Yes	1.89	(1.07 – 3.34)
Depth		
Superficial	1	
Deep	1.52	(0.879 – 2.62)
Site		
Extremities	1	
Central	0.888	(0.600 – 1.31)
Head and neck	0.599	(0.280 – 1.28)
Radiotherapy		
No	1	
Yes	1.17	(0.790 – 1.74)
Chemotherapy		
No	1	
Yes	0.674	(0.374 – 1.22)
Margin		
R0	1	
R1	1.11	(0.746 – 1.66)
R2	2.00	(1.00 – 4.01)

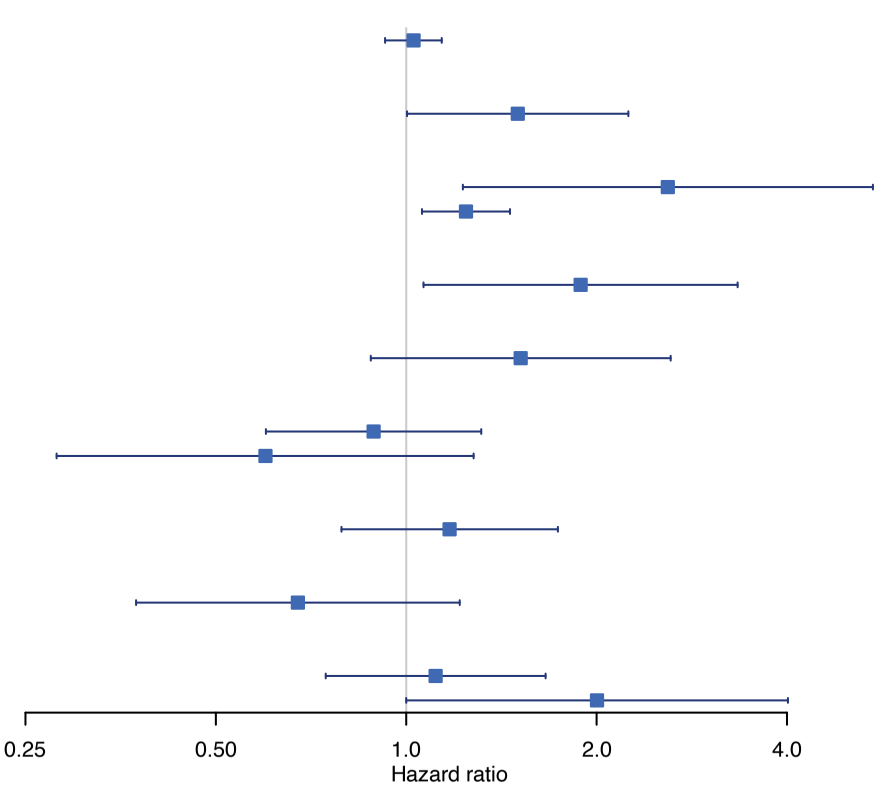


Figure 3. Multivariable cause-specific model for distant metastasis.

Square represents the HR. End of horizontal line represents 95% CI. HR: hazard ratio, CI: confidence interval, NF1: neurofibromatosis type 1

Covariates	HR	95%CI
Age (per 10 years)	1.01	(0.900–1.13)
NF1		
No	1	
Yes	2.56	(1.68–3.90)
Grade		
Low grade	1	
High grade	1.13	(0.460–2.79)
Tumor size (per 5 cm)	1.04	(0.870–1.24)
Triton		
No	1	
Yes	1.09	(0.570–2.08)
Depth		
Superficial	1	
Deep	1.19	(0.600–2.35)
Nr of metastasis sites		
1 site	1	
2 or more sites	2.10	(1.02–4.34)
Metastasis site		
Pulmonary	1	
Extra–pulmonary	0.900	(0.520–1.54)
Other	0.380	(0.160–0.890)
Metastasis–free time		
Less or equal to 1 year	1	
More than 1 year	1.28	(0.810–2.01)
Synchronous	0.890	(0.550–1.44)
Metastasectomy		
No	1	
Yes	0.240	(0.140–0.410)
Chemotherapy		
No	1	
Yes	0.550	(0.340–0.880)

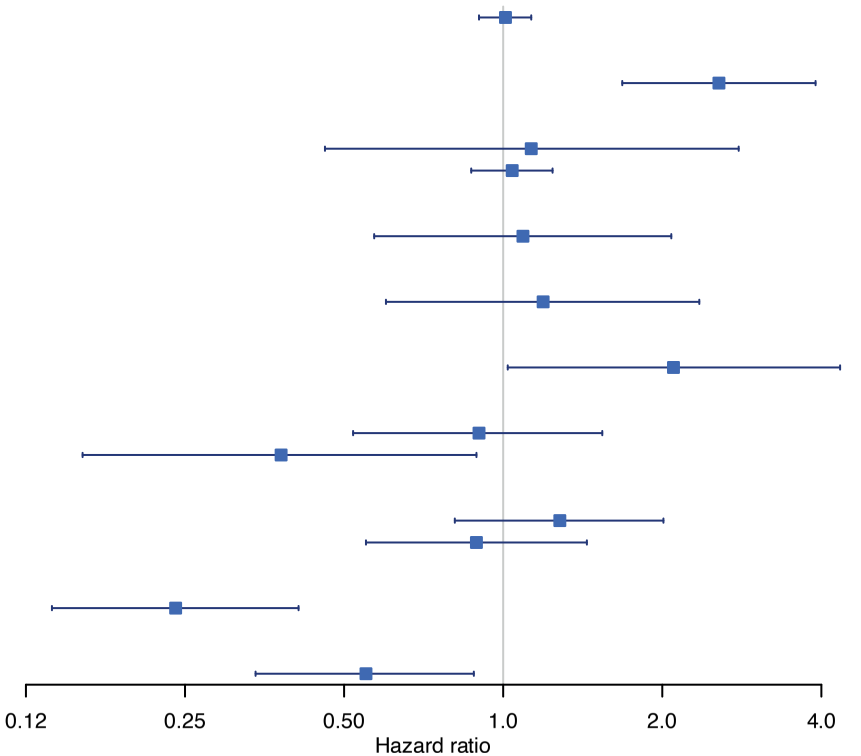


Figure 4. Multivariable Cox model on overall survival after first distant metastasis.

Square represents the HR. End of horizontal line represents 95% CI. HR: hazard ratio, CI: confidence interval, NF1: neurofibromatosis type 1

Discussion

The present study aimed to identify clinicopathologic and treatment-related factors associated with the development of DM and with OS after DM. No differences in clinicopathologic characteristics and clinical outcomes were found between patients with synchronous and metachronous DM. NF1, high grade, tumour size, triton, and R2 resections were independent risk factors for the development of DM. NF1, and more than 2 metastasis sites were independently associated with worse OS after DM. Metastasectomy, chemotherapy and the metastatic site category 'other' were associated with better survival after DM.

Risk factors for the development of metastatic disease in primary MPNST

Consistent with the literature, this study demonstrated that size is an important prognostic factor for the development of DM in primary MPNST. (22-27) Site of the primary tumour and depth do not seem to be an independent risk factor for the development of DM. (22-27) However, literature review yields some contradictory results for the factors NF1, grade, triton, and R2 resection.

In **Table 4** an overview of previous large (N>100) cohort studies published after 2000 has been depicted. Seven out of eight studies assessed the effect of NF1 on DM. Five studies did not find a significant association between NF1 and DM. Some studies concluded that NF1-associated MPNST was not perse associated with worse outcome but had more adverse clinicopathological characteristics such as larger tumours, which might explain worse clinical outcomes. (24, 27) However, the largest and most recent studies, including this study, revealed that NF1 is an independent risk factor for DM, independent of site, depth, grade, size, and surgical margin. (23) The association between triton tumours and DM was only assessed in one other study. (24) In univariable analysis the association between triton and DM was significant but in multivariable analysis this association disappeared. Further studies are needed to better understand differences in tumour biology and clinical outcome in NF1-associated MPNST and triton tumours vs sporadic MPNST, and how this could be translated to optimal management of MPNST. Surgical margin was assessed in six studies. Studies in which surgical margin was categorized as positive vs negative, no difference in DM risk was observed. However, studies in which the R classification was used, R2 resection was associated with higher risk of DM in uni- or multivariable analysis. Therefore, the R classification seems more informative than a dichotomous classification of surgical margin in MPNST.

Table 4. Overview of common predictors of DM in previous large (n > 100) cohort studies

Study	N	Analysis	5-year DMFS/ 5-year DM-rate	Factors influencing risk of DM[a]						
				NF1	Site	Depth	Grade	Size	Triton	R2
Current study	383	MV	49.8/30.5	+	NS	NS	+	+	+	+
Xu et al 2021[b]	764	MV	NR/NR	*NA*	NS	*NA*	NS*	+	*NA*	*NA*
Miao et al 2019	251	MV	60.6/NR	+	NS*	NS*	NS	+	*NA*	NS*
Watson et al 2017[c]	225	MV	49.6/NR	NS	NS	NS*	*NA*	+	NS[g]*	NS[d]
LaFemina et al 2013	105	UV	NR/NR	NS	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
Stucky et al 2012[c]	175	UV	NR/NR	NS	NS	NS	+	+	*NA*	+
Zou et al 2009	113	MV	NR/37-69[f]	NS*	NS	*NA*	*NA*	+	*NA*	NS[d]
Anghileri et al 2006	205	MV	NR/26.2	NS	NS	*NA*	+	+	*NA*	NS[d]

N: number of patients, UV: univariable analyses, MV: multivariable analyses, DMFS: distant metastasis-free survival, DM-rate: distant metastasis rate, DM: distant metastasis, NF1: neurofibromatosis type 1, NR: not reported

[a] significantly associated with lower DM risk (-), significantly associated with higher DM risk (+), not significantly associated (NS), not evaluated (NA)

[b] logistic regression on risk of DM at presentation

[c] high grade MPNST

[d] surgical margin defined as positive vs negative

[e] Pearson's chi-square/Fisher's exact test used

[f] 5-yr DM rate in patients with and without NF1 was 37% and 69% respectively (death as competing risk not taken into account)

[g] Sporadic MPNST vs epithelioid type or triton tumour

* significant in univariable analysis

Risk factors for overall survival in metastatic MNPST

To the authors' knowledge, this is the only study to date assessing prognostic factors for OS in synchronous and metachronous metastatic MPNST. One study assessed prognostic factors for OS in patients with synchronous metastasis only based on the SEER database. (22) However, this study was unable to assess the effect of DFI on OS and did not include MPNST specific information such as NF1-status. As only one study assessed OS after DM in MPNST, we made an overview of previous large (n>100) cohort studies assessing OS after DM in all STS subtypes (**Appendix Table 3**). In accordance with most of the studies, size and depth of the primary tumour do not seem to be associated with OS after DM. (5, 6, 9, 10, 13, 22, 28-31) However, the prognostic value of number of metastases or number of metastatic site and DFI has been subject of debate. Five studies, including this study, found an association between number of metastases or number of metastatic sites and worse OS after DM, while 6 studies did not find an association. (5, 9, 10, 13, 22, 29-33) Furthermore, the association between DFI and OS seems inconsistent between studies. Five studies did not find an association between the DFI and OS, while 8 studies found a significant

association. (5-7, 13, 30-35) Interestingly, 5 out of 6 studies of STS patients after pulmonary metastasectomy found a significant association between DFI and OS. It seems that the longer the DFI is, the better the OS after metachronous DM is. (5-7, 30, 31, 33, 35) This trend, although not significant, is also observed in our study. However, some studies showed worse OS in synchronous metastasis compared with metachronous metastasis, while others showed better OS in synchronous metastasis. (5, 32, 35) In our study, MPNST patients with synchronous metastasis do not seem to represent a more aggressive subgroup of tumours compared with patients who initially presented with nonmetastatic disease and experienced a DM at a later point in time. However, we only included patients with synchronous metastasis who received surgery for the primary tumour. Patients with synchronous metastasis who did not receive surgery for the primary tumour are likely to have poorer outcomes.

Even though some older and smaller studies did not find an association between NF1 and OS, recent studies conclude that NF1 is associated with worse OS. (23, 36) This multicentre study reveals that besides the higher risk for DM, NF1 is also independently associated with worse OS after DM. This might be explained by the higher risk of the development of second malignancies in MPNST patients with NF1 (37), or by a more aggressive tumour biology in NF1-associated metastatic MPNST. This underlines the potential added value of MPNST-specific information in prognostic tools and in clinical decision-making.

Treatment of metastatic MPNST

The optimal management of patients with metastatic MPNST is an important field of research. Palliative systemic therapy is the standard treatment in widespread metastatic disease. (8) However, metastasectomy is recommended in isolated resectable lung metastases (with a DFI ≥ 1 year), if complete excision of the lesions is feasible. (8) Especially in the metastatic setting, the anticipated side effects of these treatment modalities should be well balanced with the expected benefits. In our series, CTX, mainly monotherapy doxorubicin, was the most frequently offered treatment for synchronous and first metachronous disease followed by metastasectomy. However, the actual percentage of CTX in synchronous metastasis might be higher as we only included patients who were surgically treated for the primary tumour. Patients with second metachronous metastasis received mainly best supportive care.

Metastasectomy was the most important prognostic factor for better OS. The 2-year survival in patients with and without metastasectomy was 57.1% and 14.3%, respectively, in accordance with other studies. (10, 22, 31, 34, 35) Furthermore, this study found a significant association between systemic treatment and better OS in metastatic MPNST with a 2-year survival difference of 11.6% between patients

with and without CTX. The improved survival after metastasectomy and CTX is most likely due to selection bias, as a selected group of patients with a in general overall better health status, mainly receive these treatment options. Therefore, careful decision-making, taking all prognostic factors into consideration, is critical.

Strengths and limitations

This multicentre retrospective study has some inevitable limitations due to its retrospective design. Selective loss of follow-up and missing data might lead to selection bias. However, more than 90% of our study population was followed until death, and multiple imputation technique was used to reduce this risk of bias. Furthermore, no central review of pathology was performed. The diagnosis of MPNST can be challenging due to the lack of specific histologic criteria. A French cohort showed that after systematic review 20% of the MPNSTs, mainly sporadic MPNSTs, were misclassified as MPNST (38). Therefore, some MPNSTs might have been misclassified, which is an inherent limitation to all sarcoma studies without central pathology review.

However, to our knowledge, this is the first nationwide study on metastatic MPNST to date including MPNST specific information. This design prevents selection bias and allows us to make inferences on the epidemiology of metastatic MPNST in an unselected patient population. As STS is a heterogeneous group of malignancies, research on single histological subtypes is vital to improve our understanding of tumour behaviour, facilitate patient-tailored decision-making, and find a right balance between quantity and quality of life. Unlike most population-based studies on (metastatic) MPNST, this study included important entity-specific information, such as NF1- and triton-status, and included clinicopathologic information on metachronous metastasis, and follow-up.

Conclusion

Almost 40% of the MPNST patients develop DM within five years. There are no differences in clinicopathological factors and oncological outcomes between synchronous and metachronous metastasis. High grade and R2 resections are mainly associated with the development of DM. Besides that, NF1-status is associated with a higher risk of DM, this is the first study that reveals that NF1-status is also independently associated with a worse survival in metastatic MPNST, with a median survival difference of more than 6 months.

References

1. Zagars GK, Ballo MT, Pisters PW, Pollock RE, Patel SR, Benjamin RS, et al. Prognostic factors for patients with localized soft-tissue sarcoma treated with conservation surgery and radiation therapy: an analysis of 1225 patients. Cancer. 2003;97(10):2530-43.
2. Acem I, Verhoef C, Rueten-Budde AJ, Grünhagen DJ, van Houdt WJ, van de Sande MAJ. Age-related differences of oncological outcomes in primary extremity soft tissue sarcoma: a multistate model including 6260 patients. European journal of cancer (Oxford, England : 1990). 2020;141:128-36.
3. Coindre JM, Terrier P, Guillou L, Le Doussal V, Collin F, Ranchère D, et al. Predictive value of grade for metastasis development in the main histologic types of adult soft tissue sarcomas: a study of 1240 patients from the French Federation of Cancer Centers Sarcoma Group. Cancer. 2001;91(10):1914-26.
4. Bedi M, King DM, Charlson J, Whitfield R, Hackbarth DA, Zambrano EV, et al. Multimodality management of metastatic patients with soft tissue sarcomas may prolong survival. Am J Clin Oncol. 2014;37(3):272-7.
5. Lochner J, Menge F, Vassos N, Hohenberger P, Kasper B. Prognosis of Patients with Metastatic Soft Tissue Sarcoma: Advances in Recent Years. Oncol Res Treat. 2020;43(11):613-9.
6. Verschoor AJ, Litière S, Marréaud S, Judson I, Toulmonde M, Wardelmann E, et al. Prognostic relevance of distant metastases versus locally advanced disease in soft tissue sarcomas: An EORTC-STBSG database study. European journal of cancer (Oxford, England : 1990). 2018;94:187-98.
7. Italiano A, Mathoulin-Pelissier S, Cesne AL, Terrier P, Bonvalot S, Collin F, et al. Trends in survival for patients with metastatic soft-tissue sarcoma. Cancer. 2011;117(5):1049-54.
8. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2021;32(11):1348-65.
9. Cariboni U, De Sanctis R, Giaretta M, Voulaz E, Morenghi E, Colombo P, et al. Survival Outcome and Prognostic Factors After Pulmonary Metastasectomy in Sarcoma Patients: A 18-Year Experience at a Single High-volume Referral Center. Am J Clin Oncol. 2019;42(1):6-11.
10. Ferguson PC, Deheshi BM, Chung P, Catton CN, O'Sullivan B, Gupta A, et al. Soft tissue sarcoma presenting with metastatic disease: outcome with primary surgical resection. Cancer. 2011;117(2):372-9.
11. Sleijfer S, Ouali M, van Glabbeke M, Krarup-Hansen A, Rodenhuis S, Le Cesne A, et al. Prognostic and predictive factors for outcome to first-line ifosfamide-containing chemotherapy for adult patients with advanced soft tissue sarcomas: an exploratory, retrospective analysis on large series from the European Organization for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group (EORTC-STBSG). European journal of cancer (Oxford, England : 1990). 2010;46(1):72-83.
12. Smith R, Pak Y, Kraybill W, Kane JM, 3rd. Factors associated with actual long-term survival following soft tissue sarcoma pulmonary metastasectomy. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2009;35(4):356-61.
13. Iqbal N, Shukla NK, Deo SV, Agarwala S, Sharma DN, Sharma MC, et al. Prognostic factors affecting survival in metastatic soft tissue sarcoma: an analysis of 110 patients. Clin Transl Oncol. 2016;18(3):310-6.

14. Kane JM, Finley JW, Driscoll D, Kraybill WG, Gibbs JF. The treatment and outcome of patients with soft tissue sarcomas and synchronous metastases. Sarcoma. 2002;6(2):69-73.
15. Miettinen MM, Antonescu CR, Fletcher CDM, Kim A, Lazar AJ, Quezado MM, et al. Histopathologic evaluation of atypical neurofibromatous tumors and their transformation into malignant peripheral nerve sheath tumor in patients with neurofibromatosis 1—a consensus overview. Hum Pathol. 2017;67:1-10.
16. Brooks JS, Freeman M, Enterline HT. Malignant "Triton" tumors. Natural history and immunohistochemistry of nine new cases with literature review. Cancer. 1985;55(11):2543-9.
17. Coindre JM. Grading of soft tissue sarcomas: review and update. Arch Pathol Lab Med. 2006;130(10):1448-53.
18. Saklad M. GRADING OF PATIENTS FOR SURGICAL PROCEDURES. Anesthesiology. 1941;2(3):281-4.
19. Neurofibromatosis: Conference Statement. Arch Neurol. 1988;45(5):575-8.
20. R Development Core Team. R: A language and environment for statistical computing. 3.6.3 ed. Vienna, Austria: R Foundation for Statistical Computing; 2010.
21. Rubin DB. Multiple Imputation After 18+ Years. Journal of the American Statistical Association. 1996;91(434):473-89.
22. Xu Y, Xu G, Liu Z, Duan J, Lin Y, Zhu J, et al. Incidence and prognosis of distant metastasis in malignant peripheral nerve sheath tumors. Acta Neurochir (Wien). 2021;163(2):521-9.
23. Miao R, Wang H, Jacobson A, Lietz AP, Choy E, Raskin KA, et al. Radiation-induced and neurofibromatosis-associated malignant peripheral nerve sheath tumors (MPNST) have worse outcomes than sporadic MPNST. Radiother Oncol. 2019;137:61-70.
24. Watson KL, Al Sannaa GA, Kivlin CM, Ingram DR, Landers SM, Roland CL, et al. Patterns of recurrence and survival in sporadic, neurofibromatosis Type 1-associated, and radiation-associated malignant peripheral nerve sheath tumors. J Neurosurg. 2017;126(1):319-29.
25. Stucky C-CH, Johnson KN, Gray RJ, Pockaj BA, Ocal IT, Rose PS, et al. Malignant peripheral nerve sheath tumors (MPNST): the Mayo Clinic experience. Annals of surgical oncology. 2012;19(3):878-85.
26. Zou C, Smith KD, Liu J, Lahat G, Myers S, Wang W-L, et al. Clinical, pathological, and molecular variables predictive of malignant peripheral nerve sheath tumor outcome. Ann Surg. 2009;249(6):1014-22.
27. Anghileri M, Miceli R, Fiore M, Mariani L, Ferrari A, Mussi C, et al. Malignant peripheral nerve sheath tumors: prognostic factors and survival in a series of patients treated at a single institution. Cancer. 2006;107(5):1065-74.
28. Carbonnaux M, Brahmi M, Schiffler C, Meeus P, Sunyach MP, Bouhamama A, et al. Very long-term survivors among patients with metastatic soft tissue sarcoma. Cancer Med. 2019;8(4):1368-78.
29. Savina M, Le Cesne A, Blay JY, Ray-Coquard I, Mir O, Toulmonde M, et al. Patterns of care and outcomes of patients with METAstatic soft tissue SARComa in a real-life setting: the METASARC observational study. BMC Med. 2017;15(1):78.
30. Dossett LA, Toloza EM, Fontaine J, Robinson LA, Reed D, Druta M, et al. Outcomes and clinical predictors of improved survival in a patients undergoing pulmonary metastasectomy for sarcoma. Journal of surgical oncology. 2015;112(1):103-6.
31. Canter RJ, Qin LX, Downey RJ, Brennan MF, Singer S, Maki RG. Perioperative chemotherapy in patients undergoing pulmonary resection for metastatic soft-tissue sarcoma of the extremity : a retrospective analysis. Cancer. 2007;110(9):2050-60.

32. Chudgar NP, Brennan MF, Munhoz RR, Bucciarelli PR, Tan KS, D'Angelo SP, et al. Pulmonary metastasectomy with therapeutic intent for soft-tissue sarcoma. J Thorac Cardiovasc Surg. 2017;154(1):319-30.e1.
33. Blackmon SH, Shah N, Roth JA, Correa AM, Vaporciyan AA, Rice DC, et al. Resection of pulmonary and extrapulmonary sarcomatous metastases is associated with long-term survival. Ann Thorac Surg. 2009;88(3):877-84; discussion 84-5.
34. Basile G, Mattei JC, Alshaygy I, Griffin AM, Catton CN, Chung PW, et al. Curability of patients with lymph node metastases from extremity soft-tissue sarcoma. Cancer. 2020;126(23):5098-108.
35. Younger E, Husson O, Asare B, Benson C, Judson I, Miah A, et al. Metastatic Soft Tissue Sarcomas in Adolescents and Young Adults: A Specialist Center Experience. J Adolesc Young Adult Oncol. 2020;9(6):628-38.
36. Martin E, Coert JH, Flucke UE, Slooff W-BM, Ho VKY, van der Graaf WT, et al. A nationwide cohort study on treatment and survival in patients with malignant peripheral nerve sheath tumours. European journal of cancer (Oxford, England : 1990). 2020;124:77-87.
37. Williams LA, Moertel CL, Richardson M, Marcotte EL. Incidence of second malignancies in individuals diagnosed with malignant peripheral nerve sheath tumors. J Neurooncol. 2020;147(3):701-9.
38. Le Guellec S, Decouvelaere A-V, Filleron T, Valo I, Charon-Barra C, Robin Y-M, et al. Malignant Peripheral Nerve Sheath Tumor Is a Challenging Diagnosis: A Systematic Pathology Review, Immunohistochemistry, and Molecular Analysis in 160 Patients From the French Sarcoma Group Database. Am J Surg Pathol. 2016;40(7):896-908.

Appendix

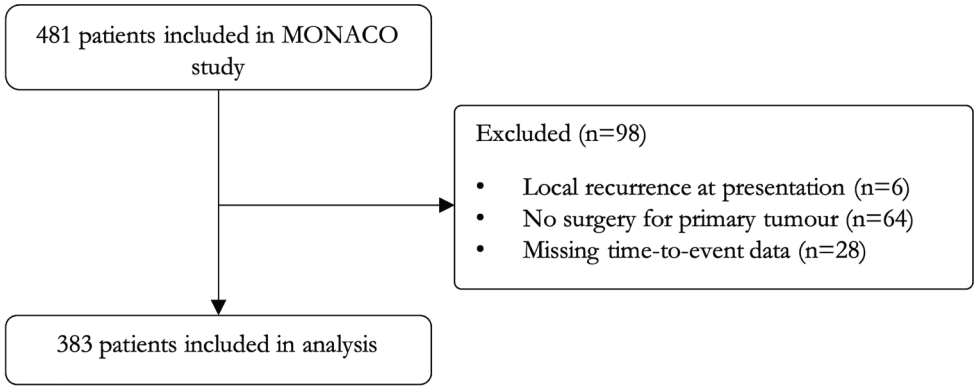


Figure 1. Consort flow diagram. n: number of patients

Table 1. baseline characteristics of the total cohort of 383 patients with primary MPNST

	Overall (N= 383)
Age (years)	
Median [IQR]	44 [29–59]
Gender	
Female	173 (45.3%)
Male	209 (54.7%)
Missing	*1*
ASA	
I	183 (56.7%)
II	123 (38.1%)
III	17 (5.26%)
Missing	*60*
Tumour size (mm)	
Median [IQR]	70.0 [40.0–112.5]
Missing	*36*
Depth	
Superficial	72 (19.8%)
Deep	291 (80.2%)
Missing	*20*
Grade	
Low grade	52 (13.6%)
High grade	329 (86.4%)
Missing	*2*
Site	

Extremities	157 (41.3%)
Central	179 (47.1%)
Head and neck	44 (11.6%)
Missing	*3*
NF1	
No	258 (68.3%)
Yes	120 (31.7%)
Missing	*5*
Within neurofibroma	
No	329 (86.4%)
Yes	52 (13.6%)
Missing	*2*
Triton	
No	348 (91.8%)
Yes	31 (8.2%)
Missing	*4*
RTX-associated	
No	357 (94.9%)
Yes	19 (5.1%)
Missing	*7*
Margin	
R0	193 (52.7%)
R1	131 (35.8%)
R2	42 (11.4%)
Missing	*17*
Radiotherapy	
No	179 (47.2%)
Yes	200 (52.8%)
Missing	*4*
Chemotherapy	
No	331 (86.6%)
Yes	51 (13.4%)
Missing	*1*

N: number of patients, IQR: Interquartile range, ASA: American Society of Anaesthesiologists Physical Status, NF1: Neurofibromatosis type 1, RTX: radiotherapy

Table 2. Baseline characteristics in patients with synchronous vs metachronous metastasis

	Synchroon (n=36)	Metachroon (n=114)	Overall (N=150)
Age (years)			
Median [IQR]	48 [25–58]	42 [28–57]	44 [27–84]
ASA			
I	17 (56.7%)	53 (54.6%)	70 (55.1%)
II	12 (40.0%)	38 (39.2%)	50 (39.4%)
III	1 (3.3%)	6 (6.2%)	7 (5.5%)
Missing	*6*	*17*	*23*
Tumour size (mm)			
Median [IQR]	105 [71–150]	80 [60–145]	90 [60–146]
Missing	*2*	*12*	*14*
Depth			
Superficial	1 (2.8%)	16 (15.2%)	17 (12.1%)
Deep	35 (97.2%)	89 (84.8%)	124 (87.9%)
Missing	*0*	*9*	*9*
Grade			
Low grade	0 (0%)	8 (7.1%)	8 (5.4%)
High grade	36 (100%)	105 (92.9%)	141 (94.6%)
Missing	*0*	*1*	*1*
Site			
Extremities	16 (44.4%)	54 (47.4%)	70 (46.7%)
Central	19 (52.8%)	51 (44.7%)	70 (46.7%)
Head and neck	1 (2.8%)	9 (7.9%)	10 (6.7%)
NF1			
No	23 (65.7%)	68 (60.2%)	91 (61.5%)
Yes	12 (34.3%)	45 (39.8%)	57 (38.5%)
Missing	*1*	*1*	*2*
Neurofibroma			
Not in neurofibroma	33 (94.3%)	97 (85.8%)	130 (87.8%)
Within neurofibroma	2 (5.7%)	16 (14.2%)	18 (12.2%)
Missing	*1*	*1*	*2*
Triton			
No	34 (94.4%)	99 (88.4%)	133 (89.9%)
Yes	2 (5.6%)	13 (11.6%)	15 (10.1%)
Missing	*0*	*2*	*2*
RTX-associated			
No	35 (97.2%)	105 (92.9%)	140 (94.0%)
Yes	1 (2.8%)	8 (7.1%)	9 (6.0%)
Missing	*0*	*1*	*1*
Site of metastasis			
Pulmonary only	11 (30.6%)	27 (23.9%)	38 (25.5%)
Extra-pulmonary (±lung)	19 (52.8%)	70 (61.9%)	89 (59.7%)
Other	6 (16.7%)	16 (14.2%)	22 (14.8%)
Missing	*0*	*1*	*1*
Number of metastatic sites			
1 site	29 (80.6%)	91 (80.5%)	120 (80.5%)
2 or more sites	7 (19.4%)	22 (19.5%)	29 (19.5%)
Missing	*0*	*1*	*1*

N: number of patients, IQR: Interquartile range, ASA: American Society of Anaesthesiologists Physical Status, NF1: Neurofibromatosis type 1, RTX: radiotherapy

Table 3. Overview of common predictors of OS after DM diagnosis in previous large (n>100) cohort studies

Study	N	Analysis	Population	OS after DM diagnosis	Factors influencing OS after DM diagnosis [a]									
					NF1	Size (pri)	Grade	Triton	Depth (pri)	Number of metastatic sites	Number of metastases	DFI	Metastasectomy	CTX
Current	150	MV	Metastatic MPNST	2-yr: 23.9% 5-yr: 13.2% Median: 8.9 months	+	NS	NS	NS	NS	+	NA	NS	-	-
Xu 2021	109	MV	Synchronous metastatic MPNST	2-yr: 14.4% Median: 8 months	NA	NS	NS	NA	NA	NA	+	NA	-	NS[b]
Basile 2020	120	UV	STS with LNM	5-yr: 57.3%[c]	NA	NA	NA	NA	NA	NA	NA	NS	-	NA
Lochner 2020	212	MV	Metastatic STS	5-yr: 24.8% Median: 24 months	NA	+	NS	NA	NA	NS	NA	-[d]	NA	NA
Younger 2020	455	MV	Metastatic STS in AYA	5-yr: 16% Median: 19.2 months	NA	NA	NA	NA	NA	NA	NA	-[e]	-	+[f]
Cariboni 2019	154	MV	STS and BS after PM	5-yr: 36.5% Median: 35.4 months	NA	NA	NS	NA	NA	NA	NS	NS	NA	NA
Verschoor 2018	1202	MV	STS with DM only	Median: 10.6 months	NA	NA	NS	NA	NA	NA	NA	-[g]	NA	NA
Carbonnaux 2018	409	MV[h]	Metastatic STS	Median: 12.1 months	NA	NS	+	NA	NA	NA	NA	NS	NA	NA
Chudgar 2017	539	MV	STS after PM	5-yr: 34% Median: 33.2 months	NA	+	NS	NA	NA	NA	+	+/-[i]	NA	+[j]
Savina 2017	1575	MV	Metastatic STS who received at least one systemic treatment	Median: 12.5 months [k]	NA	NA	+	NA	NA	NS	NA	NA	NA	-[f]
Iqbal 2016	110	MV	Metastatic STS	2-yr: 19% Median: 10 months	NA	+	NS	NA	NA	NS	NA	NS	NA	NA
Dossett 2015	120	MV	STS and BS after PM	5-yr: 44% Median: 17.2 months	NA	NA	NS	NA	NA	NA	NS	-[l]	NA	NA
Ferguson 2011	112	UV	Synchronous metastatic STS	5-yr: 17%	NA	NS	NS	NA	NS	NA	+	NA	-	NS

Study	N	Analysis	Population	OS after DM diagnosis	Factors influencing OS after DM diagnosis [a]									
					NF1	Size (pri)	Grade	Triton	Depth (pri)	Number of metastatic sites	Number of metastases	DFI	Metastasectomy	CTX
Italiano 2011	1024	MV	Metastatic STS	2-yr: 33.2% Median: 14 months	NA	NA	+	NA	NA	NA	NA	-[m]	NA	NA
Blackmon 2009	234	UV	STS and BS after PM	5-yr: 26% Median: 36.2	NA	NA	NA	NA	NA	+	NA	-[n]	NA	NA
Canter 2007	138	MV	STS after PM	Median: 30 months [o]	NA	NS	NA	NA	NA	NA	NS	-[p]	-	NS

N: number of patients, UV: univariable analyses, MV: multivariable analyses, OS: overall survival, DM: distant metastasis, NF1: neurofibromatosis type 1, pri: primary tumour, No: number, DFI: Disease-free interval/Time until metastasis, CTX: chemotherapy, MPNST: malignant peripheral nerve sheath tumour, STS: soft tissue sarcoma, LNM: lymph node metastasis, AYA: adolescents and young adults, BS: bone sarcoma, PM: pulmonary metastasis

[a] significantly associated with lower risk of death (-), significantly associated with higher risk of death (+), not significantly associated (NS), not evaluated (NA)

[b] Not specified whether CTX for primary or metastatic disease

[c] 5-year OS for patients with isolated LNM

[d] Synchronous metastasis had a worse OS compared with metachronous metastasis > 12 months

[e] Synchronous metastasis had a worse OS compared with metachronous metastasis

[f] Polychemotherapy in first line

[g] DFI of ≥2 years associated with better OS

[h] Logistic regression analysis for 5-yr survival. Censoring not taken into account

[i] Patients with synchronous or metachronous metastasis > 12 months had a better OS compared with patients with a metachronous metastasis <6 months

[j] Patients with response to CTX had a worse OS compared with patients with no CTX and patients with no response to CTX

[k] Median OS of MPNST specific

[l] DFI of > 12 months associated with better OS compared with ≤12 months

[m] Patients with DFI ≥ 36 months had a better OS compared with patients with metachronous metastasis <36 months, but no difference in OS compared with patients with synchronous metastasis

[n] DFI analyzed as continuous variable

[o] Median disease specific survival

[p] DFI of > 1 year was associated with better OS

Chapter 4

Local Recurrence in Malignant Peripheral Nerve Sheath Tumours: A Multicentre Cohort Study

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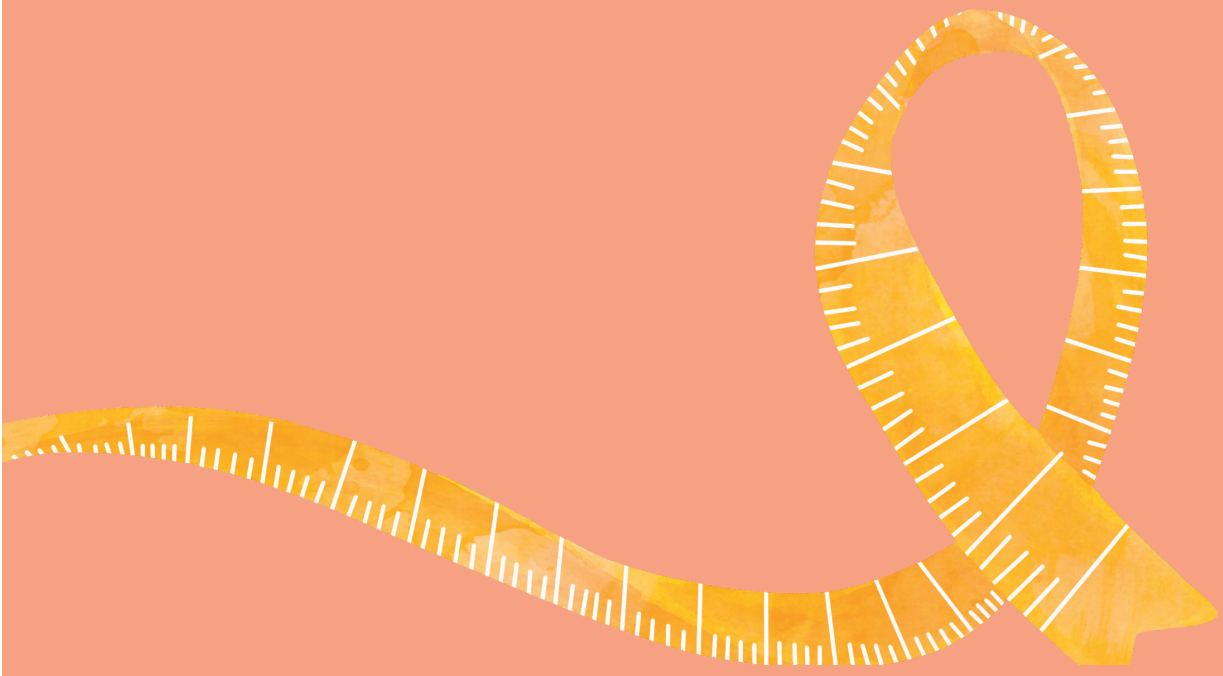
Accepted by BJS Open



Abstract

Background

Malignant Peripheral Nerve Sheath Tumours (MPNSTs) are rare, aggressive soft-tissue sarcomas (STS) with high local recurrence (LR) rates. Risk factors and optimal LR treatment vary in literature due to rarity. This study aims to elucidate treatment options and risk factors for first and second LRs (LR1 and LR2) in a large multicentre cohort.

Method

Patients with surgically treated primary MPNSTs between 1988 and 2019 in the MONACO multicentre cohort were included. Cox proportional hazard regression models were used to analyse risk factors for LR1, LR2, and overall survival (OS) after LR1. Treatment of LR1 and LR2 were evaluated.

Results

Among 507 patients, 28% developed an LR1. Median follow-up was 66.9 months, and for survivors 111.1 months. Independent LR1 risk factors included high-grade tumours (HR 2.63; 95% CI, 1.15-5.99), microscopically positive margins (HR 2.19; 95% CI, 1.51-3.16), and large tumour size (HR 2.14; 95% CI, 1.21-3.78). Peri-operative radiotherapy was associated with a lower risk of LR1 (HR 0.62; 95% CI, 0.43-0.89). LR1 patients had poorer OS than patients without an LR. Synchronous metastasis was associated with poorer OS (HR 1.79; 95% CI 1.02-3.14) post-LR1, while surgically treated LRs were associated with a better OS (HR 0.38; 95% CI 0.22-0.64) compared to non-surgical cases. Two-year survival after surgical treatment was 71% (95% CI 63%-82%) versus 28% (95% CI 18%-44%) for non-surgically-treaded LR1 patients. Most LR1 (75.4%) and LR2 (73.7%) patients received curative-intent treatment, often surgery alone (64.9% vs. 47.4%). Radiotherapy combined with surgery was given to 11.3% of LR1 and 7.9% of LR2 patients.

Conclusion

MPNST patients with a large, high-grade or R1 resection are at higher risk for the development of LR1. This risk could be potentially reduced by radiotherapy. Surgically treated recurrences exhibit improved overall survival.

Introduction

Malignant Peripheral Nerve Sheath Tumours (MPNSTs) are rare and aggressive malignant soft tissue sarcomas (STS) and compromise 5-10% of all STS. (1-3) Approximately 50% of MPNSTs arise sporadically, while about 25-50% of MPNST cases are associated with neurofibromatosis type 1 (NF1). (4-10) Patients with NF1 have an increased risk of developing an MPNST with a lifetime risk of 8-13%. (4, 11-16) MPNSTs can originate within a (plexiform) neurofibroma in patients with NF1 and can also be presented with partial rhabdomyoblastic differentiation (Triton tumour). (17) In addition, MPNSTs can also develop sporadically or be associated with prior exposure to radiation. (4, 18) Considering the various potential tumour locations, MPNSTs can exhibit a range of diverse clinical presentations. According to the European Society for Medical Oncology (ESMO) guidelines, the cornerstone of treatment for primary MPNST remains surgery with the aim of achieving clear surgical margins and therefore increasing survival. (19) While there are no recommended adjuvant treatments for MPNSTs, perioperative radiotherapy (RTX) is often used to improve local control. (1, 6, 20) On the other hand, the role of perioperative chemotherapy (CTX) has not yet been fully defined. Conflicting results have been reported in the literature regarding survival benefits of CTX. Despite complete resection and the use of RTX, studies show that about 30-70% of MPNST patients experience a first LR (LR1). (7, 18, 20-22) With these numbers, MPNSTs harbour among the highest recurrence rates in STS. (23) Due to its rarity, risk factors for the development of an LR1 vary in current literature. In **Appendix Table 1**, an overview of previous larger cohort studies assessing predictors for LR has been depicted. The development of an LR1 in patients is associated with a morbid event that decreases functional outcomes. (24) Since many patients have already undergone multimodality treatment (i.e., surgery and RTX) before experiencing a recurrence, the management of the recurrence is consequently associated with higher morbidity. (25) In certain cases, achieving local control after an LR1 may be more challenging than with primary tumours, primarily due to the distorted anatomy resulting from previous treatment. (26) There is significant value in identifying risk factors and investigating the present treatment approaches and outcomes for recurrent cases. Overall, a diagnosis of MPNST carries a poor prognosis, and in the current literature, the treatment of recurrences remains unclear and varies. (1, 4, 7, 18, 27, 28) The primary objective of treatment of recurrence is to prolong disease-free survival, nevertheless, second recurrences (LR2) do occur. Therefore, it is crucial to understand the impact of treatment options for an LR1 on the development of an LR2 and on overall survival (OS) after an LR1.

The aim of this project is to identify risk factors associated with recurrence, and the treatment of recurrences, as well as their impact on OS in MPNST patients across nine

sarcoma centres in The Netherlands and the Mayo Clinic. Additionally, we aimed to characterize the risk factors related to the development of an LR2 and treatment of an LR2.

Methods

Patient Population

A retrospective cohort study of the nine Dutch sarcoma centres and the Mayo Clinic, the MONACO study, was undertaken after approval of the institutional review boards of the participating centres. All patients diagnosed with pathologically proven primary MPNST from 1988 to 2019 who were surgically treated for the primary tumour were included in this study. Follow-up was done according to nationwide guidelines. The diagnosis of all patients conformed to the World Health Organization's classification of soft tissue and bone tumours. (29) Patients with uncertain pathological reports or diagnoses based on incomplete information during follow-up were excluded. Additionally, patients who presented with local recurrence after previous resection at a different facility were excluded from the study.

Covariates

Covariates extracted from medical records for analysis were patient, tumour, and treatment characteristics and survival data. AN LR1 was defined as the first radiological or pathological evidence of a recurrence at the site of the primary tumour bed. AN LR2 was defined as the second radiological or pathological evidence of a recurrence at the site of the first recurrence. Age was determined as the patient's age at the time of diagnosis. The American Society of Anaesthesiologist (ASA) classification system was employed to categorize patients' physical status. (30) Tumour size was assessed as the maximum diameter of the tumour mass through imaging or pathology reports. Tumour grade was categorized as either low- or high-grade based on the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system. Tumours originating below or within the deep fascia were classified as deep-seated. NF1 status was extracted from pathological reports and was established either when explicitly mentioned in the report or when there was a pathology report of previous plexiform neurofibroma resections or the presence of two or more neurofibromas.

Surgical margin was categorized as R0 (microscopically negative, no tumour cells found in surgical borders), R1 (microscopically positive) or R2 (macroscopically positive). Tumour site was divided into extremity, central (including thorax, abdomen, pelvis, retroperitoneal), and head and neck categories. Triton status was extracted from pathological reports and was confirmed either when explicitly mentioned or when the report indicated MPNST with rhabdomyoblastic differentiation. RTX-associated

MPNST was defined as having previously received radiation therapy at the same site as the primary tumour bed. The study's endpoints included LR1, LR2, and OS.

Statistical analysis

All statistical analyses were performed in R (version 4.2.2). Baseline characteristics as well as treatment modalities were compared between patients with and without an LR1 and LR2 during follow-up.

Overall survival was defined as the duration from definitive surgery to either the date of death or the date of the last follow-up. Time-to-LR was defined as the time interval between definitive surgery and date of first LR. Time-to-LR2 was defined as the time interval between LR1 and date of LR2. Estimated median survival was calculated using the Kaplan-Meier method for several covariates of interest.

Multivariable Cox Proportional Hazards (PH) models were used to estimate the effect of several covariates on the development of an LR1, OS after the LR1, and the development of an LR2. In the multivariate models with LR1 or LR2 as primary outcome, death was considered as a competing risk. The selection of candidate predictors for the various outcomes was based on clinical expertise and existing literature. Univariable and multivariable analyses with 95% confidence intervals (CI) were used to estimate the effects of the covariates on the different outcomes. Variables with a p-value < 0.25 from the univariable analyses were included for further evaluation when constructing the multivariable model.

Proportional hazards were assessed visually with the Schoenfeld residuals. Missing values were imputed using multiple imputations (MI) (m = 20), and estimates were pooled using Rubin's rule. (31) A p-value ≤ 0.05 was considered statistically significant. Results from the Cox PH models were described in hazard ratios (HR) with 95% CI. All statistical tests were two-sided. The packages 'mice' for MI, 'survival', 'rms' and 'survminer' were used for the survival and competing risk analyses.

Results

Patient population

A total of 755 patients were included in the MONACO database. Patients who presented with a metastasis at presentation (n = 102), who were not treated surgically for the primary tumour (n = 49), who had an R2 resection (n = 76), with missing data on LR1 (n = 12), and patients with incomplete time-to-event information (n = 9) were excluded in this analysis (**Figure 1**). Of the 507 patients included in this study, 142 developed an LR1 during the follow-up period. Of the 142 patients with an LR1,

patients without treatment for their recurrence (n = 50), patients with a metastasis during their LR1 (n = 13), patients with an R2 margin (n = 1) and patients with missing data on their LR2 (n = 7) were excluded from further analysis.

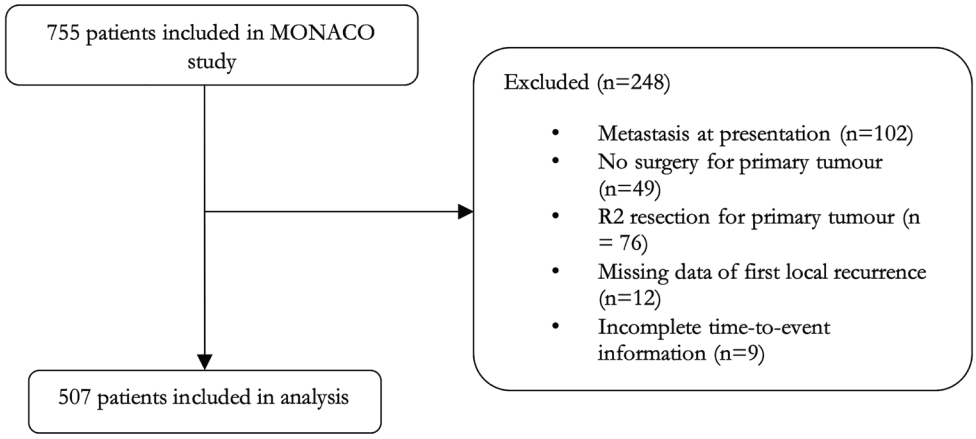


Figure 1. Study Flow chart
N: number of patients

Patient and tumour characteristics are summarized in **Table 1**. The median follow-up time for all patients was 66.9 months (IQR 108.7). The median follow-up time for survivors was 111.1 months (IQR 123.1). There was a trend for a higher incidence of NF1 in patients with an LR1 (40.0% vs 31.1%). In LR1 patients, there was a slight male prediction (51.4%). Tumours were usually large (≥5cm, 53.5%), and most were located in the extremities (50.3%). Microscopically positive margins (R1) were more common in patients with an LR1 (39.4% vs 33.2%). Patients with an LR1 were mostly treated with surgery only for their primary tumour (39.5%) or surgery and adjuvant RTX (45.9%) (**Table 2**).

Table 1. Patient characteristics of 138 MPNST patients with a first local recurrence

Variable	Overall (n=507)	No LR1 (n=365)	LR1 (n=142)
Age (years)			
Mean (SD)	43.3 (19.5)	43.2 (20.3)	43.43 (17.6)
Male gender	270	197 (54.1%)	73 (51.4%)
Missing	*1*	*1*	-
ASA			
I	160	120 (59.1%%)	40 (51.3%)
II	107	73 (36.0%)	34 (43.6%)
III	14	10 (4.9%)	4 (5.1%)
Missing	*226*	*162*	*64*
NF1			

Variable	Overall (n=507)	No LR1 (n=365)	LR1 (n=142)
No	322	241 (68.9%)	81 (60.0%)
Yes	163	109 (31.1%)	54 (40.0%)
Missing	*22*	*15*	*7*
Tumour size			
<5 cm	130	113 (37.5%)	17 (17.0%)
5-10 cm	164	117 (38.9%)	47 (47.0%)
≥10 cm	107	71 (23.6%)	36 (36.0%)
Missing	*106*	*64*	*42*
Tumour depth			
Superficial	73	58 (25.4%)	15 (17.4%)
Deep	241	170 (74.6%)	71 (82.6%)
Missing	*193*	*137*	*56*
Tumour grade			
High grade	284	201 (83.4%)	83 (92.2%)
Low grade	47	40 (16.6%)	7 (7.8%)
Missing	*176*	*124*	*52*
Triton tumour			
No	303	219 (91.2%)	84 (95.5%)
Yes	25	21 (8.8%)	4 (4.5%)
Missing	*179*	*125*	*54*
RTX-associated			
No	444	325 (93.4%)	119 (88.1%)
Yes	39	23 (6.6%)	16 (11.9%)
Missing	*24*	*17*	*7*
Site of primary tumour			
Head and neck	71	58 (6.0%)	15 (10.6%)
Extremities	255	184 (50.7%)	71 (50.0%)
Central	177	121 (33.3%)	56 (39.4%)
Missing	*21*	*21*	-
Metastasis during LR1			
No	475	365 (100.0%)	110 (77.5%)
Yes	32	0	32 (22.5%)
Surgical margin			
R0	328	257 (74.7%)	71 (55.9%)
R1	143	87 (25.3%)	56 (44.1%)
Missing	*36*	*21*	*15*
Reresection for primary tumour			
No	365	254 (75.8%)	111 (83.5%)
Yes	113	81 (24.2%)	32 (22.5%)
Missing	*39*	*30*	*9*

LR1: first local recurrence, n: number of patients, SD: standard deviation, ASA: The American Society of Anaesthesiologist classification system, NF1: neurofibromatosis type 1, RTX: radiotherapy

Table 2. Initial treatment

Variables	Overall (n=507)	No LR1 (n=365)	LR1 (n=142)
Total treatment			
Surgery alone	192	137 (41.6%)	55 (40.4%)
Surgery + RTX	223	160 (43.8%)	63 (46.3%)
Surgery + CTX	18	14 (3.8%)	4 (2.9%)
Surgery + RTX + CTX	53	39 (10.7%)	14 (10.3%)
Surgery with missing in (neo)adjuvant therapies	21	15	6
Any type of radiotherapy			
No	210	151 (42.7%)	59 (43.4%)
Yes	280	203 (57.3%)	77 (56.6%)
Missing	*17*	*11*	*6*
Pre- or postoperative radiotherapy			
No	210	151 (43.1%)	59 (44.0%)
nRTX	74	61 (17.4%)	13 (9.7%)
aRTX	200	138 (39.4%)	62 (46.3%)
Missing	*23*	*15*	*8*
Any type of chemotherapy			
No	419	298 (84.9%)	121 (87.1%)
Yes	71	53 (15.1%)	18 (12.9%)
Missing	*17*	*14*	*3*
Pre- or postoperative chemotherapy			
No	419	298 (84.9%)	121 (87.1%)
nCTX	44	37 (10.5%)	7 (5.8%)
aCTX	25	14 (4.0%)	11 (9.1%)
Both	2	2 (0.6%)	-
Missing	*17*	*14*	*3*
Primary wound closure			
No	41	35 (14.6%)	6 (6.7%)
Yes	287	204 (85.4%)	83 (93.3%)
Non-functional reconstruction			
No	386	277 (82.9%)	109 (87.2%)
Yes	73	57 (17.1%)	16 (12.8%)
Missing	*48*	*31*	*17*
Functional reconstruction			
No	444	322 (95.3%)	122 (97.6%)
Yes	19	16 (4.7%)	3 (2.4%)
Missing	*44*	*27*	*17*

LR1: first local recurrence, n: number of patients, RTX: radiotherapy, CTX: chemotherapy, nRTX: neoadjuvant radiotherapy, aRTX: adjuvant radiotherapy, nCTX: neoadjuvant chemotherapy, aCTX: adjuvant chemotherapy

Risk Factors for the development of an LR1 in primary MPNST

One-hundred-forty-two patients (28.0%) developed an LR1 after they underwent surgery for their primary tumour. The median time to an LR1 was 10.6 months (IQR 16.7). On multivariable analysis, factors independently associated with the development of an LR1 were a high tumour grade (HR 2.63; 95% CI, 1.15-5.99), microscopically positive margins (R1) (HR 2.19; 95% CI, 1.51-3.16), and a tumour size ≥5cm (HR 2.14; 95% CI, 1.21-3.78) (Table 3). On the contrary, the use of RTX (HR 0.62; 95% CI, 0.43-0.89) was associated with a reduced risk for development of an LR1.

Table 3. Univariate and multivariable analysis of risk factors for the development of a first local recurrence

	Univariable		Multivariable	
Variables	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Age (per 10 years)	1.01 (0.928-1.11)	0.767		
NF1				
No	1	-	1	-
Yes	1.49 (1.04-2.13)	0.030	1.14 (0.779-1.66)	0.507
Tumour grade				
Low grade	1	-	1	-
High grade	2.38 (1.10-5.16)	0.032	2.63 (1.15-5.99)	0.026
Tumour size				
<5 cm	1	-	1	-
≥5 cm	2.45 (1.47-4.08)	0.001	2.14 (1.21-3.78)	0.011
Triton				
No	1	-		
Yes	0.683 (0.271-1.73)	0.424		
Tumour depth				
Superficial	1	-	1	-
Deep	1.41 (0.841-2.37)	0.198	1.07 (0.607-1.90)	0.807
Site of primary tumour				
Head and neck	1	-	1	-
Extremities	1.26 (0.717-2.21)	0.425	1.10 (0.593-2.03)	0.768
Central	1.65 (0.934-2.93)	0.087	1.28 (0.682-2.38)	0.447
Margin primary tumour				
R0	1	-	1	-
R1	2.06 (1.45-2.93)	<0.001	2.19 (1.51-3.16)	<0.001
Radiotherapy primary tumour				
No	1	-	1	-
Yes	0.809 (0.544-1.14)	0.230	0.616 (0.426-0.892)	0.012
Chemotherapy primary tumour				
No	1	-		
Yes	0.897 (0.544-1.48)	0.669		

HR: hazard ratio, CI: confidence interval, NF1: neurofibromatosis type 1

Treatment of LR1

Of the patients developing an LR1, 92 (64.9%) patients were surgically treated for their recurrence (Table 4). R0 resections were achieved in 37 (37.8%) patients. R1 resections were achieved in 13 (13.3%) patients, and three patients had an R2 margin (3.1%) as final surgical margin. First local recurrences were mainly treated with surgery only (50.7%). In 29 (20.4%) patients with an LR1, no treatment was performed. Out of the 59 (41.5%) LR1 patients without primary RTX, 15 (25.4%) patients still underwent RTX for their LR1. Of the patients treated with RTX, 2.8% of patients received neoadjuvant and 14.8% adjuvant RTX to surgery. In total, 5.6% of patients received only RTX as treatment for their recurrence.

Table 4. Treatment of recurrences

Variable	LR1 (n=142)	LR2 (n=38)
Time to local recurrence		
Mean (SD)	23.3 (35.0)	17.6 (19.4)
Surgery for LR1/LR2		
No	44 (32.4%)	14 (37.8%)
Yes	92 (67.6%)	23 (62.2%)
Missing	*6*	*1*
Surgical margin		
R0	37 (37.8%)	8 (21.1%)
R1	13 (13.3%)	3 (7.9%)
R2	3 (3.1%)	1 (2.6%)
No surgery	44 (45.9%)	14 (68.4%)
Missing	*46*	*12*
Treatment of LR1/LR2		
No treatment	29 (21.3%)	9 (24.3%)
Surgery	72 (52.9%)	18 (48.6%)
Surgery + RTX	16 (11.8%)	3 (8.1%)
Surgery + CTX	3 (2.2%)	1 (2.7%)
Surgery + RTX + CTX	1 (0.7%)	1 (2.7%)
RTX	8 (5.9%)	4 (10.8%)
CTX	7 (5.1%)	1 (2.7%)
Missing	*6*	*1*
Radiotherapy		
No	65 (72.2%)	13 (61.9%)
nRTX	4 (4.4%)	-
aRTX	21 (23.3%)	8 (38.1%)
Missing	*52*	*17*
Chemotherapy		
No	80 (87.9%)	21 (87.5%)

Variable	LR1 (n=142)	LR2 (n=38)
nCTX	3 (3.3%)	1 (4.2%)
aCTX	7 (7.7%)	-
Both	1 (1.1%)	2 (8.3%)
Missing	*51*	*14*

LR1: first local recurrence, LR2: second local recurrence, n: number of patients, SD: standard deviation, RTX: radiotherapy, CTX: chemotherapy, nRTX: neoadjuvant radiotherapy, aRTX: adjuvant radiotherapy, nCTX: neoadjuvant chemotherapy, aCTX; adjuvant chemotherapy

Risk Factors for Overall Survival in MPNST patients with an LR1

The median survival from diagnosis of an LR1 till death or last follow-up date was 39.2 months (95% CI 22.3-60.0) (**Figure 2**). Out of the 142 patients with an LR1, 32 (22.5%) also had a concurrent metastasis. On multivariable analysis, factors independently associated with OS in patients with an LR1 consisted of only a metastasis during the recurrence (HR 1.79; 95% CI 1.02-3.14). Surgical treatment, on the other hand, improves OS in patients with a local recurrence (HR 0.38; 95% CI 0.22-0.64) (**Table 5**). The median survival in patients surgically treated for their LR was 56 months, compared to 43 months in patients without surgery for their LR.

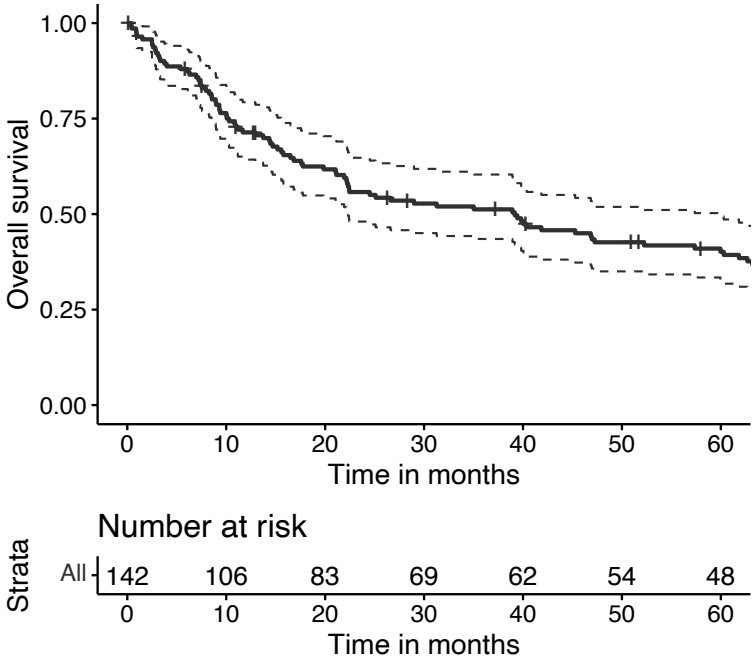


Figure 2. Survival plot of survival after first Local Recurrence (LR1)

Table 5. Univariable and multivariable analysis of risk factors for overall survival in patients with a first local recurrence

	Univariable		Multivariable	
Variables	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Age (per 10 years)	1.05 (0.938-1.17)	0.415		
NF1				
No	1	-		
Yes	0.98 (0.614-1.53)	0.938		
Tumour grade				
Low grade	1	-	1	-
High grade	2.35 (1.42-3.88)	0.001	2.06 (0.846-5.00)	0.121
Tumour size				
<5 cm	1	-	1	-
≥5 cm	1.66 (0.815-3.38)	0.170	1.24 (0.59-2.61)	0.573
Tumour depth				
Superficial	1	-	1	-
Deep	2.30 (1.42-3.71)	0.001	1.98 (0.985-3.96)	0.061
Site of primary tumour				
Head and neck	1	-		
Extremities	1.13 (0.570-2.25)	0.723		
Central	1.37 (0.681-2.74)	0.382		
Margin primary tumour				
R0	1	-	1	-
R1	1.35 (0.880-2.07)	0.174	1.08 (0.654-1.78)	0.769
Radiotherapy primary tumour				
No	1	-		
Yes	1.27 (0.830-1.94)	0.275		
Surgery LR1				
No	1	-	1	-
Yes	0.364 (0.238-0.557)	<0.001	0.375 (0.221-0.636)	<0.001
Margin LR1				
R0	1	-		
R1	1.39 (0.743-2.62)	0.307		
R2	0.777 (0.425-1.42)	0.418		
Radiotherapy LR1				
No	1	-	1	-
nRTX	0.465 (0.275-0.784)	0.005	1.34 (0.526-3.40)	0.545
aRTX	0.583 (0.325-1.05)	0.075	0.752 (0.388-1.46)	0.404
Metastasis during LR1				
No	1	-	1	-
Yes	2.532 (1.62-3.97)	<0.001	1.79 (1.02-3.14)	0.046

HR: hazard ratio, CI: confidence interval, NF1: neurofibromatosis type 1, LR1: first local recurrence, nRTX: neoadjuvant radiotherapy, aRTX: adjuvant radiotherapy

Risk factors for the development of a LR2 and treatment

A total of 71 patients were treated with curative intent for their LR1. Among these, 38 (53.5%) patients who underwent surgical treatment for their LR1 experienced an LR2 (Table 6). The median time from the surgical treatment of an LR1 to the development of an LR2 was 17.6 months (IQR 16.1). Out of the patients who developed an LR2, 32 (84.2%) were solely treated with surgery for their LR1. A total of 12 patients also received RTX following their surgery for their LR1. Among these 12 patients, 4 (33.3%) patients developed an LR2. Various potential risk factors for the development of an LR2 were analysed univariabely (Table 7). However, on univariable analysis, no statistically significant risk factors contributing to the occurrence of an LR2 could be identified.

Of the patients developing an LR2, 23 (60.5%) patients were surgically treated for their recurrence (Table 4). R0 resections were achieved in 8 (21.1%) patients. R1 resections were achieved in 3 (7.9%) patients, and one patient had an R2 margin (2.6%) as final surgical margin. Second local recurrences were mainly treated with surgery only (47.4%). In nine patients (23.7%) with an LR2, no treatment was performed. RTX combined with surgery was administered in 3 (7.9%) patients and RTX alone in 4 (10.5%).

Table 6. Patient characteristics of patients with and without a second local recurrence after surgically treated first local recurrence

Variable	Overall (n = 71)	No LR2 (n = 33)	LR2 (n = 38)
Age (years)			
Mean (SD)	42.4 (15.6)	40.2 (15.3)	44.3 (15.8)
Male gender	34	19 (57.6%)	15 (39.5%)
ASA			
I	27	14 (70.0%)	13 (68.4%)
II	11	5 (25.0%)	6 (31.6%)
III	1	1 (5.0%)	-
Missing	*32*	*13*	*19*
NF1			
No	44	22 (68.8%)	22 (61.1%)
Yes	24	10 (31.2%)	14 (38.9%)
Missing	*3*	*1*	*2*
Tumour size			
<5cm	11	5 (21.7%)	6 (25.0%)
5-10 cm	10	5 (21.7%)	5 (20.8%)
≥10 cm	26	13 (56.5%)	13 (54.2%)
Missing	*24*	*10*	*14*

Variable	Overall (n = 71)	No LR2 (n = 33)	LR2 (n = 38)
Tumour depth			
Superficial	10	4 (18.2%)	6 (28.6%)
Deep	33	18 (81.8%)	15 (71.4%)
Missing	*28*	*11*	*17*
Tumour grade			
High grade	41	22 (95.7%)	19 (82.6%)
Low grade	5	1 (4.3%)	4 (17.4%)
Missing	*25*	*10*	*15*
Triton tumour			
No	44	23 (100.0%)	21 (95.5%)
Yes	1	-	1 (4.5%)
Missing	*26*	*10*	*16*
Site of primary tumour			
Head and neck	6	1 (3.0%)	5 (13.2%)
Extremities	44	23 (69.7%)	21 (55.3%)
Central	21	9 (27.3%)	12 (31.6%)
Surgical margin LR1			
R0	29	18 (81.8%)	11 (57.9%)
R1	12	4 (18.2%)	8 (42.1%)
Missing	*30*	*11*	*19*
Treatment of LR1			
Surgery*	55	23 (69.7%)	32 (84.2%)
Surgery + RTX	12	8 (24.2%)	4 (10.5%)
Surgery + CTX	3	1 (3.0%)	2 (5.3%)
Surgery + RTX + CTX	1	1 (3.0%)	-
Radiotherapy for LR1			
No	33	14 (60.1%)	19 (82.6%)
nRTX	2	1 (4.3%)	1 (4.4%)
aRTX	11	8 (34.8%)	3 (13.0%)
Missing	*25*	*10*	*15*
Chemotherapy for LR1			
No	40	21 (91.3%)	19 (90.5%)
nCTX	3	1 (4.3%)	2 (9.5%)
Both	1	1 (4.3%)	0 (0%)
Missing	*24*	*10*	*14*

* Patients who received surgery alone or with unknown (neo)adjuvant treatment. LR2: second local recurrence, n: number of patients, SD: standard deviation, ASA: The American Society of Anaesthesiologist classification system, NF1: neurofibromatosis type 1, LR1: first local recurrence, RTX: radiotherapy, CTX: chemotherapy, nRTX: neoadjuvant radiotherapy, aRTX: adjuvant radiotherapy, nCTX: neoadjuvant chemotherapy

Table 7. Univariable analysis of risk factors for the development of a second local recurrence

Variables	Univariable HR (95% CI)	p-value
Tumour grade		
Low grade	1	
High grade	0.627 (0.262-1.50)	0.308
Tumour size		
<5 cm	1	
≥5 cm	1.01 (0.376-2.69)	0.991
Site of primary tumour		
Head and neck	1	
Extremities	0.404 (0.150-1.08)	0.081
Central	0.605 (0.213-1.72)	0.352
Margin LR1		
R0	1	
R1	2.01 (0.832-4.87)	0.140
Radiotherapy LR1		
No	1	
nRTX	1.05 (0.376-2.92)	0.930
aRTX	0.373 (0.111-1.25)	0.125
Chemotherapy LR1		
No	1	
nCTX	1.17 (0.389-3.49)	0.786
Both	1.10 (0.398-3.02)	0.861

HR: hazard ratio, CI: confidence interval, LR1: first local recurrence, nRTX; neoadjuvant radiotherapy, aRTX: adjuvant radiotherapy, nCTX: neoadjuvant chemotherapy

Discussion

This study aimed to characterize treatment and outcomes of local recurrences in patients with MPNSTs and to identify risk factors for recurrence, treatment, and predictors of OS after LR1. A high grade, large tumour size (≥5 centimetre), and microscopically positive margins were independent risk factors for the development of an LR1. The administration of RTX for the primary tumour reduced the risk of the development of an LR1. The treatment of LRs varied, and most patients were treated with surgery alone or with surgery and RTX. Synchronous metastasis during a local recurrence had a negative impact on OS, while a surgically treated recurrence is expected to improve overall survival.

Risk Factors for the development of a LR1 in primary MPNST

Among all types of sarcomas, MPNSTs have one of the highest recurrence rates. (32) However, in current literature, only a small number of papers identified risk factors for the development of an LR1 in MPNST. According to the literature (**Appendix Table 1**), a high tumour grade, microscopically positive margins and a large tumour size are important prognostic factors for the occurrence of an LR1 in MPNST patients, which is consistent with the findings of this study. (21, 33, 34) The importance of surgical quality seems crucial in the development of a recurrence. A study demonstrates that poor surgical margins for primary tumours have a significant impact on local control and a minor effect on metastasis-free survival and cause-specific mortality. (35)

One study suggests that trunk wall MPNSTs yield a higher risk for LR, however, this was not seen in this study. (33) While NF1 has been associated with worse prognosis, most likely due to a higher biological risk for metastasis, it does not seem to be a risk factor for LR. (5, 17) However, studies have reported that patients with NF1 are more likely to have larger tumours, which are associated with LR. (11, 33)

While contradictory results have been reported in literature regarding the use of RTX in patients with MPNSTs, the current study demonstrates that RTX is associated with a reduced risk of developing an LR1. (7, 11, 32, 33, 36-38) This finding is in line with the use of RTX in other types of STS. (39, 40) According to the NCCN and ESMO guidelines, RTX is recommended in the treatment of STS when achieving a complete (R0) resection is not feasible, as well as in cases of a high-grade STS. (19, 41) In STS, both neoadjuvant RTX and adjuvant RTX have shown to provide equal levels of local control. (42-44) However, in current literature, there is still some discussion in the use of RTX in patients with MPNSTs when an R0 resection is expected. (32, 45, 46)

Overall Survival after Local Recurrence

MPNSTs have been associated with poor prognosis, with five-year survival rates ranging from 40.6% to 61.9%. (7, 8, 12, 37, 38, 45, 47, 48) In the literature, it remains unclear what the actual impact of local recurrences is on OS and what prognostic factors are relevant for STS patients, including MPNST patients. (49) Clinical investigations have been conducted on survival after LR1 in more frequent types of STS. (50, 51) However, significant factors that affect survival after an LR1 are still unknown for MPNSTs. Several clinical and pathological variables could have a significant effect in predicting survival after a recurrence. The occurrence of concomitant metastasis during an LR1 was independently associated with worse OS following the LR1 diagnosis. This observation is consistent with the findings of another paper focused on primary STS. (50) In the study, all types of STS were included of which 6.5% were MPNSTs. However, further investigation is needed to explore the

relationship between histologic subtypes and LRs, as it is reasonable to assume that tumours with different subtypes may demonstrate distinct clinical behaviours and modified survival outcomes. (50)

In this cohort, a microscopically positive margin was not identified as an independent risk factor for OS after LR diagnosis. Also, tumour grade did not emerge as a significant prognostic factor influencing survival in MPNST patients after LR1. However, it should be acknowledged that the findings of this study may have been affected by a limited number of cases involving low-grade tumours (7 out of 142). In contrast to other retrospective studies, tumour size was not identified as a significant factor. (12, 13, 33, 45) The variability in the chosen cut-offs observed in other published cohorts, ranging from 5 to 15 cm, could possibly explain this discrepancy. The use of RTX did not have a significant influence on survival in our study. The current literature on the use of RTX still presents inconclusive results. Some studies demonstrate improved survival in patients receiving RTX, while others do not show improved long-term survival. (6, 12, 36, 45, 47, 52-54)

A recurrence that has been treated surgically is expected to improve the 2-year survival in patients diagnosed with an LR1. This is in line with one other large cohort study (n = 477) in which complete surgical resection of the tumour is a significant prognostic factor for patients with recurrent STS. (50)

Treatment of Local Recurrences in MPNST

The occurrence of an LR1 after prior resection, with or without RTX, significantly impacts patients' well-being. Managing an LR1 becomes challenging due to the complexities of prior therapies and recurrence in a previously irradiated area. The treatment of recurrences depends on several factors, including the patient's physical condition, preferences, and the feasibility of curative interventions. The feasibility of a curative treatment depends on various tumour characteristics, one of which is the presence of concomitant metastasis, which is a poor prognostic factor as shown in this study. One study states that the occurrence of an LR1 is strongly influenced by the feasibility of surgical intervention for the primary tumour. (55) However, these results could be hampered by indication bias since patients were more likely to be selected for surgery based on tumour and patient characteristics.

For primary MPNSTs, surgical resection is the recommended treatment, aiming to achieve complete removal with clear margins as the primary objective. (22) Although adjuvant or neoadjuvant therapy is being increasingly considered, its effectiveness in improving survival in primary MPNSTs has not been consistently demonstrated. (48)

As discussed above, according to the ESMO and NCCN guidelines, the standard of care for primary STS is surgery combined with RTX. (19, 41) The NCCN guideline suggests that for patients with recurrent STS, treatment decisions should follow the same algorithm as for patients with a new primary lesion. If an LR1 can be excised, the decision to use re-irradiation should be made on a case-by-case basis due to varying outcomes reported in the literature. (41) Although MPNSTs generally exhibit more aggressive behaviour than most types of STS, risk factors for the development of an LR1 in other types of STS include high grade, microscopically positive margins, and tumour size, consistent with findings in our cohort. (50, 56) This suggests that the same treatment strategy for recurrences may be applicable for recurrent MPNSTs as well. The authors suggest surgery as the primary treatment modality for patients with recurrent MPNSTs, while a personalized approach may be most effective for adjuvant treatment. When considering the use of RTX as adjuvant treatment, it is important to take into account the disadvantages, such as wound complications in preoperative RTX and late radiation toxicities in postoperative RTX. These factors should be considered in the decision-making process as they can have a negative impact on functional outcome scores in patients. (25, 57) Furthermore, it is important to consider that around 10% of MPNSTs can arise as a result of previous irradiation, particularly among NF1 patients. (58) This should also be taken into account during the decision-making process.

Despite curative treatment in patients with an LR1, there is still a high risk of developing an LR2. However, there is no literature available on risk factors for the development of an LR2 in MPNST patients, and only a small amount of papers have been published on LR2 in other types of STS. (49, 59, 60)

Approximately 54% of patients with an LR1 requiring surgical treatment develop an LR. This is consistent with an study investigating LR2 in patients with STS who underwent surgical treatment for their LR1, which reported a second recurrence rate of 50%. (59) Two other studies reported an LR2 rate ranging from 24 to 37% in patients with STS. In current study, no statistically significant predictors for the development of an LR2 in patients with an LR1 were found. Most patients with an LR2 in our study underwent surgical treatment, consistent with the literature. (49, 60)

Strengths and Limitations

This multicentre retrospective study is subject to inevitable limitations arising from its retrospective design, including potential selection bias due to selective loss of follow-up and missing data. However, over 90% of the included patients were followed until death, and multiple imputation technique was used to reduce this risk of bias. Due to its retrospective nature, patients in this study underwent treatment over a span of

nearly 30 years, potentially leading to variations in treatment standards that could impact the results. Additionally, it is important to acknowledge that a central review of pathology was not performed in this study, which could introduce limitations in accurately diagnosing MPNST due to the absence of specific histologic criteria. Also, due to the low number of patients treated for an LR1 and subsequently developing an LR2, it is likely that univariate analyses could not find any significant risk factors.

Nevertheless, due to the size of this large international and nationwide study on recurrent MPNST, new insights have been provided. Furthermore, as all included patients were treated in specialized centres, the review of pathology might be of lesser significance. This design enhances the generalizability of the data and models by minimizing the potential for selection or referral bias. As STS can present very heterogeneously, research on a single histological subtype level is necessary to improve our understanding of tumour behaviour and to aid tailoring ideal treatment and outcomes. In contrast to most population-based studies on (recurrent) MPNST, this study incorporated significant entity-specific details, including NF1- and Triton-status, as well as important clinical and treatment information on local recurrences.

Conclusion

Almost 30% of the MPNST patients develop an LR. Consistent with the literature, this study demonstrated that risk factors associated with a higher risk of a recurrence were a high grade, microscopically positive margins, and tumour size. The use of RTX is associated with a reduced risk of the development of a recurrence. The treatment of local recurrences varied, and most patients were treated with surgery only or surgery with RTX. Synchronous metastasis during an LR1 had a negative impact on OS, while surgically treated cases showed longer OS. Despite curative treatment of an LR1, 54% developed an LR2 during follow-up.

References

1. Bradford D, Kim A. Current treatment options for malignant peripheral nerve sheath tumors. Current treatment options in oncology. 2015;16:1-8.
2. Amirian ES, Goodman JC, New P, Scheurer ME. Pediatric and adult malignant peripheral nerve sheath tumors: an analysis of data from the surveillance, epidemiology, and end results program. Journal of neuro-oncology. 2014;116:609-16.
3. Ducatman BS, Scheithauer BW, Piepgras DG, Reiman HM, Ilstrup DM. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. Cancer. 1986;57(10):2006-21.
4. Du P, Zhu J, Zhang ZD, He C, Ye MY, Liu YX, et al. Recurrent epithelioid malignant peripheral nerve sheath tumor with neurofibromatosis type 1: A case report and literature review. Oncol Lett. 2019;18(3):3072-80.
5. Martin E, Coert JH, Flucke UE, Slooff WM, Ho VKY, van der Graaf WT, et al. A nationwide cohort study on treatment and survival in patients with malignant peripheral nerve sheath tumours. European journal of cancer (Oxford, England : 1990). 2020;124:77-87.
6. Zou C, Smith KD, Liu J, Lahat G, Myers S, Wang W-L, et al. Clinical, pathological, and molecular variables predictive of malignant peripheral nerve sheath tumor outcome. Annals of surgery. 2009;249(6):1014-22.
7. Sobczuk P, Teterycz P, Czarnecka AM, Świtaj T, Koseła-Paterczyk H, Kozak K, et al. Malignant peripheral nerve sheath tumors–Outcomes and prognostic factors based on the reference center experience. Surgical Oncology. 2020;35:276-84.
8. Valentin T, Le Cesne A, Ray-Coquard I, Italiano A, Decanter G, Bompas E, et al. Management and prognosis of malignant peripheral nerve sheath tumors: the experience of the French Sarcoma Group (GSF-GETO). European journal of cancer. 2016;56:77-84.
9. Enzinger FM WS. Malignant tumours of peripheral nerves. Soft Tissue Tumors. 2001(31):1209–63.
10. Edizer DT, Özdoğan A, Karaman E, Işıldak H. Report of a case of malignant peripheral nerve sheath tumor of the neck with an early local recurrence. Ear, Nose & Throat Journal. 2011;90(10):E1-E3.
11. Watson KL, Al Sannaa GA, Kivlin CM, Ingram DR, Landers SM, Roland CL, et al. Patterns of recurrence and survival in sporadic, neurofibromatosis type 1–associated, and radiation-associated malignant peripheral nerve sheath tumors. Journal of neurosurgery. 2017;126(1):319-29.
12. Miao R, Wang H, Jacobson A, Lietz AP, Choy E, Raskin KA, et al. Radiation-induced and neurofibromatosis-associated malignant peripheral nerve sheath tumors (MPNST) have worse outcomes than sporadic MPNST. Radiotherapy and Oncology. 2019;137:61-70.
13. LaFemina J, Qin L-X, Moraco NH, Antonescu CR, Fields RC, Crago AM, et al. Oncologic outcomes of sporadic, neurofibromatosis-associated, and radiation-induced malignant peripheral nerve sheath tumors. Annals of surgical oncology. 2013;20:66-72.
14. Imura Y, Outani H, Takenaka S, Yasuda N, Nakai S, Nakai T, et al. Clinical outcomes and prognostic factors for patients with malignant peripheral nerve sheath tumour. Sarcoma. 2021;2021.
15. Evans DGR, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. Journal of medical genetics. 2002;39(5):311-4.
16. Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. Journal of medical genetics. 2007;44(2):81-8.
17. Acem I, Martin E, van Houdt WJ, van de Sande MAJ, Grünhagen DJ, Verhoef C, et al. The Association of Metastasis Pattern and Management of Metastatic Disease with Oncological Out-

comes in Patients with Malignant Peripheral Nerve Sheath Tumors: A Multicenter Cohort Study. Cancers (Basel). 2021;13(20).
18. James AW, Shurell E, Singh A, Dry SM, Eilber FC. Malignant Peripheral Nerve Sheath Tumor. Surg Oncol Clin N Am. 2016;25(4):789-802.
19. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO–EURACAN–GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2021;32(11):1348-65.
20. Hagi T, Nakamura T, Yokoji A, Matsumine A, Sudo A. Medullary metastasis of a malignant peripheral nerve sheath tumor: A case report. Oncol Lett. 2016;12(3):1906-8.
21. Stark AM, Buhl R, Hugo HH, Mehdorn HM. Malignant peripheral nerve sheath tumours–report of 8 cases and review of the literature. Acta neurochirurgica. 2001;143:357-64.
22. Akshintala S, Mallory NC, Lu Y, Ballman KV, Schuetze SM, Chugh R, et al. Outcome of Patients With Malignant Peripheral Nerve Sheath Tumors Enrolled on Sarcoma Alliance for Research Through Collaboration (SARC) Phase II Trials. The Oncologist. 2023;28(5):453-9.
23. Collin C, Godbold J, Hajdu S, Brennan M. Localized extremity soft tissue sarcoma: an analysis of factors affecting survival. Journal of Clinical Oncology. 1987;5(4):601-12.
24. Eilber FC, Rosen G, Nelson SD, Selch M, Dorey F, Eckardt J, et al. High-grade extremity soft tissue sarcomas: factors predictive of local recurrence and its effect on morbidity and mortality. Annals of surgery. 2003;237(2):218.
25. Guerrero WM, Deneve JL. Local recurrence of extremity soft tissue sarcoma. Surgical Clinics. 2016;96(5):1157-74.
26. Robinson M, Barr L, Fisher C, Fryatt I, Stotter A, Harmer C, et al. Treatment of extremity soft tissue sarcomas with surgery and radiotherapy. Radiotherapy and Oncology. 1990;18(3):221-33.
27. Martin E, Geitenbeek RTJ, Coert JH, Hanff DF, Graven LH, Grünhagen DJ, et al. A Bayesian approach for diagnostic accuracy of malignant peripheral nerve sheath tumors: a systematic review and meta-analysis. Neuro Oncol. 2021;23(4):557-71.
28. Geitenbeek RTJ, Martin E, Graven LH, Broen MPG, Anten M, van der Pol JAJ, et al. Diagnostic value of (18)F-FDG PET-CT in detecting malignant peripheral nerve sheath tumors among adult and pediatric neurofibromatosis type 1 patients. J Neurooncol. 2022;156(3):559-67.
29. Board WCoTE. International Agency for Research on Cancer. 2020.
30. Saklad M, editor Grading of patients for surgical procedures. The Journal of the American Society of Anesthesiologists; 1941: The American Society of Anesthesiologists.
31. Rubin DB. Multiple imputation after 18+ years. Journal of the American statistical Association. 1996;91(434):473-89.
32. Kar M, Deo SV, Shukla NK, Malik A, DattaGupta S, Mohanti BK, et al. Malignant peripheral nerve sheath tumors (MPNST)–clinicopathological study and treatment outcome of twenty-four cases. World Journal of Surgical Oncology. 2006;4(1):1-8.
33. Anghileri M, Miceli R, Fiore M, Mariani L, Ferrari A, Mussi C, et al. Malignant peripheral nerve sheath tumors: prognostic factors and survival in a series of patients treated at a single institution. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2006;107(5):1065-74.
34. Wang T, Yin H, Han S, Yang X, Wang J, Huang Q, et al. Malignant peripheral nerve sheath tumor (MPNST) in the spine: a retrospective analysis of clinical and molecular prognostic factors. Journal of neuro-oncology. 2015;122:349-55.
35. Gronchi A, Casali PG, Mariani L, Miceli R, Fiore M, Lo Vullo S, et al. Status of surgical margins and prognosis in adult soft tissue sarcomas of the extremities: a series of patients treated at a single institution. Journal of Clinical Oncology. 2005;23(1):96-104.

36. Stucky C-CH, Johnson KN, Gray RJ, Pockaj BA, Ocal IT, Rose PS, et al. Malignant peripheral nerve sheath tumors (MPNST): the Mayo Clinic experience. Annals of surgical oncology. 2012;19:878-85.
37. Carli M, Ferrari A, Mattke A, Zanetti I, Casanova M, Bisogno G, et al. Pediatric malignant peripheral nerve sheath tumor: the Italian and German soft tissue sarcoma cooperative group. Journal of clinical oncology. 2005;23(33):8422-30.
38. Wanebo JE, Malik JM, Vandenberg SR, Wanebo HJ, Driesen N, Persing JA. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 28 cases. Cancer. 1993;71(4):1247-53.
39. Yang JC, Chang AE, Baker AR, Sindelar WF, Danforth DN, Topalian SL, et al. Randomized prospective study of the benefit of adjuvant radiation therapy in the treatment of soft tissue sarcomas of the extremity. Journal of clinical oncology. 1998;16(1):197-203.
40. Group VTM. Randomised trial of volume of post- operative radiotherapy given to adult patients with extremity soft tissue sarcoma: VORTEX; 2010 [Available from: http://www.birmingham.ac.uk/research/activity/ mds/trials/crctu/trials/vortex/index.aspx.
41. Von Mehren M, Randall RL, Benjamin RS, Boles S, Bui MM, Ganjoo KN, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network. 2018;16(5):536-63.
42. O'Sullivan B, Davis AM, Turcotte R, Bell R, Catton C, Chabot P, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. The Lancet. 2002;359(9325):2235-41.
43. Davis AM, O'sullivan B, Bell RS, Turcotte R, Catton CN, Wunder JS, et al., editors. Function and health status outcomes in a randomized trial comparing pre-operative and post-operative radiotherapy in extremity soft tissue sarcoma. Orthopaedic Proceedings; 2004: Bone & Joint.
44. Leidinger B, Heyse T, Schuck A, Buerger H, Mommsen P, Bruening T, et al. High incidence of metastatic disease in primary high grade and large extremity soft tissue sarcomas treated without chemotherapy. BMC cancer. 2006;6:1-9.
45. Fan Q, Yang J, Wang G. Clinical and molecular prognostic predictors of malignant peripheral nerve sheath tumor. Clinical and Translational Oncology. 2014;16:191-9.
46. Minovi A, Basten O, Hunter B, Draf W, Bockmühl U. Malignant peripheral nerve sheath tumors of the head and neck: management of 10 cases and literature review. Head & Neck: Journal for the Sciences and Specialties of the Head and Neck. 2007;29(5):439-45.
47. Yuan Z, Xu L, Zhao Z, Xu S, Zhang X, Liu T, et al. Clinicopathological features and prognosis of malignant peripheral nerve sheath tumor: a retrospective study of 159 cases from 1999 to 2016. Oncotarget. 2017;8(62):104785.
48. Porter DE, Prasad V, Foster L, Dall GF, Birch R, Grimer RJ. Survival in malignant peripheral nerve sheath tumours: a comparison between sporadic and neurofibromatosis type 1-associated tumours. Sarcoma. 2009;2009.
49. Abatzoglou S, Turcotte RE, Adoubali A, Isler MH, Roberge D. Local recurrence after initial multidisciplinary management of soft tissue sarcoma: is there a way out? Clinical Orthopaedics and Related Research®. 2010;468:3012-8.
50. Sugiura H, Tsukushi S, Yoshida M, Nishida Y. What is the success of repeat surgical treatment of a local recurrence after initial wide resection of soft tissue sarcomas? Clinical Orthopaedics and Related Research. 2018;476(9):1791.
51. Liang Y, Guo T, Hong D, Xiao W, Zhou Z, Zhang X. Time to local recurrence as a predictor of survival in patients with soft tissue sarcoma of the extremity and abdominothoracic wall. Frontiers in Oncology. 2020;10:599097.

52. Wong WW, Hirose T, Scheithauer BW, Schild SE, Gunderson LL. Malignant peripheral nerve sheath tumor: analysis of treatment outcome. International Journal of Radiation Oncology* Biology* Physics. 1998;42(2):351-60.
53. Kahn J, Gillespie A, Tsokos M, Ondos J, Dombi E, Camphausen K, et al. Radiation therapy in management of sporadic and neurofibromatosis type 1-associated malignant peripheral nerve sheath tumors. Frontiers in oncology. 2014;4:324.
54. Ferner RE, Gutmann DH. International consensus statement on malignant peripheral nerve sheath tumors in neurofibromatosis. AACR; 2002.
55. Bergamaschi L, Bisogno G, Manzitti C, D'Angelo P, Milano GM, Scagnellato A, et al. Salvage rates and prognostic factors after relapse in children and adolescents with malignant peripheral nerve sheath tumors. Pediatr Blood Cancer. 2018;65(2).
56. Sawamura C, Matsumoto S, Shimoji T, Okawa A, Ae K. How long should we follow patients with soft tissue sarcomas? Clinical Orthopaedics and Related Research®. 2014;472:842-8.
57. Felderhof JM, Creutzberg CL, Putter H, Nout RA, Bovée JVMG, Dijkstra PDS, et al. Long-term clinical outcome of patients with soft tissue sarcomas treated with limb-sparing surgery and postoperative radiotherapy. Acta Oncologica. 2013;52(4):745-52.
58. Farid M, Demicco EG, Garcia R, Ahn L, Merola PR, Cioffi A, et al. Malignant peripheral nerve sheath tumors. Oncologist. 2014;19(2):193-201.
59. Trovik CS, Bauer HCF, Alvegård TA, Anderson H, Blomqvist C, Berlin Ö, et al. Surgical margins, local recurrence and metastasis in soft tissue sarcomas: 559 surgically-treated patients from the Scandinavian Sarcoma Group Register. European journal of cancer. 2000;36(6):710-6.
60. Moureau-Zabotto L, Thomas L, Bui BNG, Chevreau C, Stockle E, Martel P, et al. Management of soft tissue sarcomas (STS) in first isolated local recurrence: a retrospective study of 83 cases. Radiotherapy and Oncology. 2004;73(3):313-9.

Appendix

Table 1. Overview of predictors for the development of a LR1 in cohort studies

Study	n of patients	Type of STS	Analysis	Factors influencing Risk of LR1						
				NF1	Grade	Tumor size	Depth	Site	Margin (R1)	RTX
Current study	499	MPNST	MV	NS	+	+	NS	NS	+	+
Stucky et al.	175	MPNST	UV	NS	NS	NS	NS	NS	NS	NS
Anghileri et al.	205	MPNST	MV	NS	+	+	NA	+	+	NS
Wang et al.	43	MPNST	MV	NA	+	NS	NA	NA	NS	NS

LR1: first local recurrence, n: number of patients, NF1: neurofibromatosis type 1, RTX: radiotherapy, MV: multivariate analysis, UV: univariate analysis, NS: not significant, +: significant, NA: not available

Chapter 5

Prognostic Significance of Immunohistochemical Markers and Genetic Alterations in Malignant Peripheral Nerve Sheath Tumours: A Systematic Review

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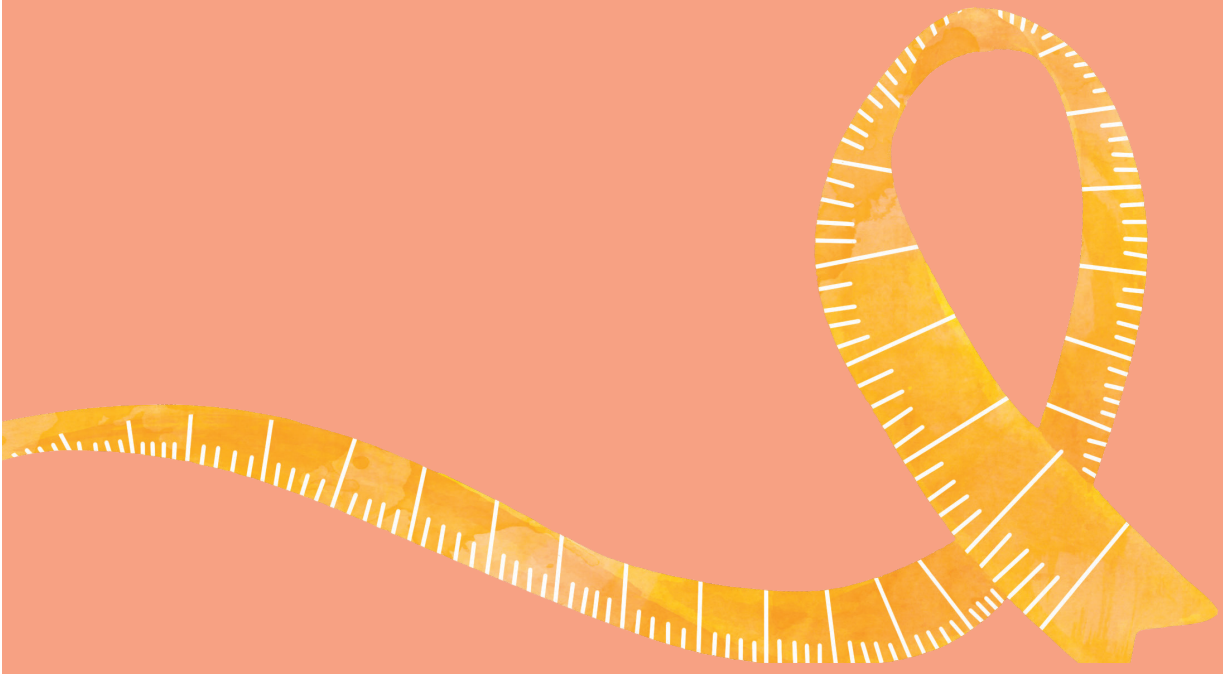

Abstract

Background

Malignant peripheral nerve sheath tumours (MPNSTs) are aggressive soft tissue sarcomas with dismal prognosis. Pathological and genetic markers may predict more aggressive behaviour in MPNSTs, but have uncommonly been investigated and few are used in daily practice. This study reviews the prognostic value of immunohistochemical markers and genetic alterations in MPNST.

Methods

A systematic search was performed in PubMed and Embase databases according to the PRISMA guidelines. Search terms related to 'MPNST' and 'prognostic' were used. Studies investigating the association of immunohistochemical markers or genetic alterations with prognosis were included. Qualitative synthesis was performed on all studies. A distinction was made between univariable and multivariable associations.

Results

Forty-six studies were included after full-text screening. Sixty-seven different immunohistochemical markers were investigated. Absence of S100 and H3K27me3 and high Ki67 and p53 staining were most commonly independently associated with worse survival and disease-free survival. Several genetic alterations were investigated as well with varying association to survival. TP53, CDK4, RASSF1A alterations were independently associated with worse survival, as well as changes in chromosomal length in Xp, 10q, and 16p.

Conclusion

MPNSTs harbour complex and heterogeneous biology. Immunohistochemical markers and genetic alterations have variable prognostic value. Absence of S100 and H3K27me3 and increased Ki67 can be of prognostic value. Alterations in TP53 or increase in p53 staining may distinguish MPNSTs with worse outcomes. Genetic alterations and staining of other cell cycle regulatory and Ras pathway proteins may also help stratifying patients with worse outcomes. A combination of markers can increase the prognostic value.

Introduction

Malignant peripheral nerve sheath tumours (MPNSTs) are rare and aggressive soft tissue sarcomas (STS) that carry a dismal prognosis. (1-3) Neurofibromatosis type 1 (NF1) patients have an increased risk of developing these tumours and encompass approximately 25-50% of MPNST patients. (1-5) The *NF1* gene is commonly affected in MPNSTs which causes loss of the neurofibromin protein which inhibits the Ras enzyme. (6) Activation of the Ras pathway leads to upregulation of the mitogen-activated protein (MAPK) and phosphoinositide 3-kinase (PI3K) pathways. (7) Besides the common knockdown of *NF1*, alterations in several genes including *TP53, SUZ12, EED, PTEN,* and *CDKN2A* as well as upregulation of several tyrosine kinases contribute to the formation of MPNST. (8-12) MPNSTs are known for harbouring complex genomic alterations, but despite our increasing understanding of underlying biology, prognosis has not ameliorated the past decades and median survival stagnates at 5-6 years. (2, 3)

Staging of MPNSTs is important to increase accuracy of outcome prediction, but it may also facilitate treatment stratification. However, the clinical American Joint Committee of Cancer (AJCC) STS staging system is less applicable in MPNST. (4, 5, 13) The histologic Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system used in STS is of prognostic value since low grade MPNST (FNCLCC grade 1) has improved survival. (2) However, only 10% of MPNSTs are grade 1 and the FNCLCC grading can likely only distinguish prognosis between grade 1 and 3. (2, 5) Moreover, the histological distinction between low-grade MPNST and benign neurofibroma with atypia is difficult as objective criteria are lacking, causing interobserver variability. In the context of NF1, the diagnosis of progression to MPNST is even more challenging. Recently, a consensus view has been published defining "atypical neurofibromatous neoplasm of uncertain biologic potential (ANNUBP)" as an intermediate lesion in NF1 patients. (14) While driver mutations are increasingly being studied, the transition of neurofibromas to MPNSTs is not yet fully understood. Clinical parameters as predictors of outcome have been studied more commonly, but independent predictors are found inconsistently. (3) Although radiation-induced MPNSTs have repeatedly been associated with worse survival, the influence of NF1 disease on survival has been subject of debate. (3, 13, 15) Better classification systems for MPNSTs are therefore urgently needed.

Currently, surgery remains the only proven treatment to improve survival. (1-3) Chemotherapy has limited effect in localized disease and its use is controversial. Some studies suggest a minor benefit in high-grade, large, and deep MPNST. (16-18) Moreover, 10-20% of patients present with metastatic or unresectable disease and up

to 50% of patients will develop metastases over time. (1-5, 13, 19) Targeted therapies are warranted, but so far none have been proven effective. (20) Immunohistochemical and genetic markers may predict more aggressive behaviour in MPNSTs, but their association with oncological outcome has uncommonly been investigated and few are yet used in daily practice for prognostication. For this reason, this systematic review set out to summarize current knowledge on the prognostic value of immunohistochemical and genetic markers. Such markers may enhance prognostication and aid in elucidating driver mutations of malignancy.

Methods

Literature search

A systematic search was performed in Embase and PubMed databases according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines, in order to identify all potentially relevant articles as of March 2020. The string was built with the help of a professional librarian using search terms related to 'MPNST' and 'prognostic'. The exact search syntaxes for PubMed and Embase are shown in **Appendix A**. Studies were included that evaluated the association of immunohistochemical markers and genetic alterations to oncological outcomes in MPNST patients. Exclusion criteria included lack of full text or studies without specific analyses fitting our inclusion criteria. The initial review was conducted by two independent authors (E.M. and I.A.). Disagreements were solved through discussion in which one additional author was involved (C.V.).

Data extraction and synthesis

Data extracted from studies included: study period, total number of patients, mean age and range, percentage NF1 patients, markers and genetic alterations investigated for prognostic value, and analyses used to identify prognosticators. For all markers and genetic alterations investigated additional information was extracted: number of patients with survival data, population with 'positive' test, oncological outcome analysed, and whether its prognostic value was corrected for common clinical prognostic factors. Whenever the marker was independently associated with outcome, the hazard ratio was noted. Common factors for which could have been adjusted in multivariable models included: age, presence of NF1, tumour size, tumour site, metastasis at diagnosis, tumour depth, tumour grade, and surgical margin. (3) All results of the predictive value of markers were presented or re-calculated to represent the marker cut-off as a negative predictor of survival. Qualitative synthesis was performed for all studies, summarizing results based on type of analysis. Immunohistochemical markers were further stratified into markers of differentiation, receptors and their ligands, Ras pathway, cell cycle regulation, p53 pathway, vascularization, and others. For each

immunohistochemical marker cumulative incidence of univariable and multivariable association to survival (disease-specific or overall) or disease-free survival (recurrence, metastasis, or both) were calculated.

Results

After removal of duplicates, a total of 1882 articles were identified in PubMed and Embase databases (**Figure 1**). Title and abstract screening resulted in 55 potentially relevant articles, of which 46 were selected for qualitative synthesis after full-text screening. Mean age differed between 11 and 50 years old (range of all patients 1-94). Prevalence of NF1 patients in study populations ranged from 0-100% (mean: 48.0%). Immunohistochemical markers were studied exclusively in 36 studies, genetic alterations in 7 studies, and both in 3 studies (**Table 1**). A total of 67 different immunohistochemical markers and numerous genetic alterations were evaluated (**Table 2, Figure 2**).

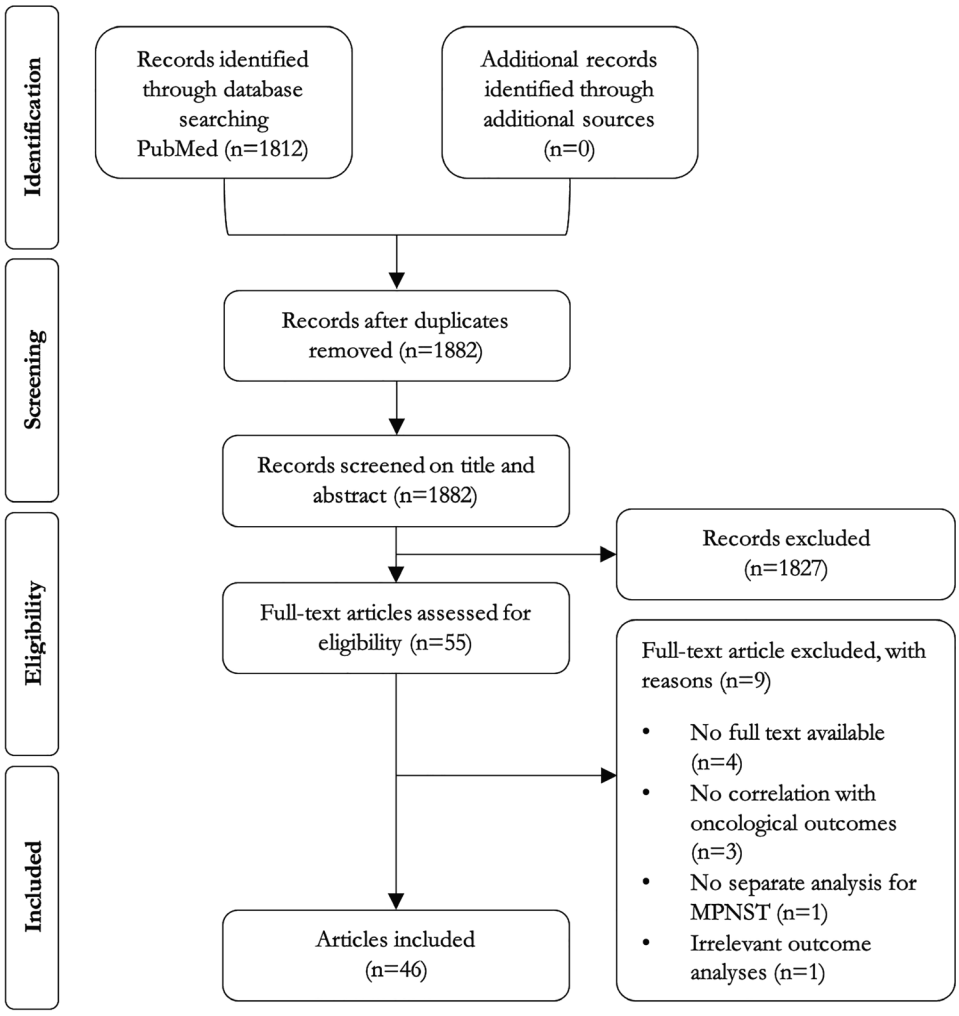

Figure 1. Flowchart of study selection

Table 1. Study characteristics of included studies

Author, year	Study period	N	Age (range)	NF1	Markers and genetic alterations	Analysis type[a]
Alaggio, 2013	1990-2007	35	11 (1-18)	42.9%	*BIRC5*	RT-PCR
Benassi, 1998	*NA*	17	*NA*	*NA*	Laminin receptor	IHC
Benassi, 2001	*NA*	15	*NA*	*NA*	MMP-2, MMP-9, TIMP-2	IHC
Brekke, 2009	1980-2002	64	41 (13-85)	43.8%	p53, p-RB, CDK2, CDK4, cyclin D1, cyclin D3, cyclin E1, p14, p16, p18, p21, p27, MDM2, Ki67	IHC
Brekke, 2010	1980-2002	48	37 (11-79)	58.3%	Xq loss, 10q loss, 16p gain, 16q loss, 5p gain, 2q gain, 6q gain, 7q gain, Xp loss, 10p loss, 4q loss, 20q gain, 1q gain	aCGH
Cleven, 2016	1979-2007	162	*NA*	49.4%	H3K27me3	IHC
Danielsen, 2015	1973-2008	91	48 (11-79)	48.4%	*RASSF1A*	Methylation-specific PCR
Endo, 2011	1964-2008	99	*NA*	33.3%	p14, p15, p16, Ki67	IHC
Endo, 2013	1964-2010	88	*NA*	40.9%	p-Akt, p-mTOR, p-S6RP, p-p70S6K, p-4E-BP1, p-MEK, p-ERK, Ki67	IHC
Fan, 2014	*NA*	56	*NA*	*NA*	MET, MDM2, p53	IHC
Fukushima, 2017	1964-2011	82	*NA*	39.0%	HIF-1α, HIF-2α, MVD, Ki67	IHC
Gong, 2018	2006-2015	14	46 (23-66)	21.4%	Ki67	IHC
Hakozaki, 2014	1992-2008	44	50 (15-86)	47.7%	COX-2	IHC
Halling, 1996	*NA*	28	39 (15-84)	50.0%	p53	IHC
Høland, 2018	1980-2010	100	36 (11-82)	50.0%	*TP53*, *MDM2*	aCGH, RT-PCR
Holtkamp, 2007	*NA*	36	40 (13-78)	61.1%	MMP-13, p53 codon	PCR, IHC
Holtkamp, 2008	*NA*	34	*NA*	76.5%	*CDKN2A*	MLPA
Ikuta, 2014	1986-2011	30	45 (17-77)	53.3%	HA, HAS1, HAS2, HAS3	IHC
Jia, 2019	2002-2011	30	49 (11-71)	*NA*	Decorin	IHC
Keizman, 2009	1994-2006	51	41 (*NA*)	51.0%	EGFR	IHC
Kobayashi, 2006	1964-2004	96	43 (0-86)	41.2%	CHFR, Ki67	IHC
Kolberg, 2015	1980-2002	63	33 (13-85)	44.4%	Survivin, TK1, TOP2A	IHC
Kourea, 1999	*NA*	35	*NA* (*NA*)	*NA*	p53, p-RB, p21, p27, cyclin D1, cyclin E, Ki67	IHC
Krawczyk, 2019	1992-2013	26	10 (*NA*)	34.6%	Survivin, cyclin D1, osteopontin, fibronectin, p53	IHC
Kresse, 2008	*NA*	7	47 (24-78)	*NA*	17q23.2-q25.3, *TOP2A*, *ETV4*, *HOXB7*, *BIRC5*, miR142p-3p, miR142-5p, miR201, miR21, miR338	aCGH, RT-PCR
LaFemina, 2013	1982-2011	105	38 (16-87)	40.0%	S100	IHC

Author, year	Study period	N	Age (range)	NF1	Markers and genetic alterations	Analysis type[a]
Le Guellec, 2016	1990-2013	124	37 (7-94)	54.8%	S100, MDM2, desmin	IHC
Leroy, 2001	1988-1999	17	32 (17-56)	100%	p53	IHC
Lu, 2018	1990-2012	74	39 (11-79)	58.1%	ATRX	IHC
Meis, 1992	1965-1985	70	10 (0-15)	20.5%	S100	IHC
Nobeyama, 2016	*NA*	20	(15-70)	100%	*MAGEA3*	Methylation-specific PCR
Otsuka, 2018	1975-2016	145	48 (1-88)	29.7%	H3K27me3	IHC
Panse, 2017	*NA*	39	*NA*	*NA*	p-STAT3	IHC
Pekmezci, 2017	1991-2012	39	37 (11-72)	66.7%	H3K27me3	IHC
Skotheim, 2003	1980-2000	51	50 (20-86)	37.3%	TOP2A, Ki67	IHC
Tabone, 2008	1985-2005	52	23 (3-60)	50.0%	EGFR	IHC
Torres, 2011	1986-2006	96	*NA*	57.3%	MET, HGF, p-MET, p53, S100	IHC
Vasconcelos, 2019	1990-2010	29	*NA* (22-83)	58.6%	MCD, MVD, Ki67	IHC
Wang, 2015	2001-2012	43	49 (*NA*)	14.0%	S100, vimentin, GFAP, NSE, Ki67, SMA, CD57	IHC
Wasa, 2008	1987-2006	22	43 (16-83)	50.0%	VEGF, MVD	IHC
Watanabe, 2001	*NA*	49	41 (17-86)	44.9%	p53, Ki67, MDM2, p21	IHC
Yu, 2011	*NA*	123	*NA*	38.2%	*SOX5, NOL1, MLF2, FOXM1, FKBP4, CDK4, TSPAN31, ERBB2, MYC, TP53*, SOX5, FOXM1, Myc, p53	aCGH, RT-PCR, FISH, IHC
Yuan, 2017	1999-2016	159	40 (5-76)	44.0%	S100, Ki67, vimentin, NF, GFAP	IHC
Zhang, 2017	1991-2011	58	47 (6-86)	0.0%	CXCR4, CXCL12, cyclin D1	IHC
Zhou, 2016	*NA*	63	*NA*	*NA*	FGFR1, FGFR2, FGFR4, *FGFR1*	FISH, IHC
Zou, 2009	1986-2006	140	35 (1-80)	51.4%	S100, Ki67, p53, VEGF, EGFR, p-MEK	IHC

[a]: used for correlation with outcome.

4E-BP1: eukaryotic translation initiation factor 4E-binding protein 1, aCGH: array-based comparative genomic hybridization, CDK: cyclin dependant kinase, CHFR: checkpoint with forkhead-associated domain and ring finger, COX-2: cyclooxygenase-2, CXCR4: C-X-C motif chemokine receptor 4, CXCL12: C-X-C motif chemokine ligand 12, EGFR: epidermal growth factor receptor, ERK: extracellular signal-regulated kinases, FISH: fluorescence in situ hybridization, FGFR: fibroblast growth factor receptor, FOXM1: forkhead box protein M1, GFAP: glial fibrillary acidic protein, H3K27me3: trimethylation of lysine 27 of histone H3, HA: hyaluronan, HAS: hyaluronan synthase, HIF: hypoxia-inducible factor, IHC: immunohistochemistry, MCD: mast cell density, MDM2: mouse double minute 2 homolog, MEK: mitogen-activated protein kinase kinase, Met: metastasis, MLPA: multiplex ligation-dependent probe amplification, MMP-13: matrix metallopeptidase 13, mTOR: mammalian target of rapamycin, MVD: microvessel density, N: total number of patients, NA: not available, NF1: neurofibromatosis type 1p-: phosphorylated, RASSF1A: Ras association domain family member 1, isoform A, RT-PCR: reverse transcription polymerase chain reaction, S6RP: ribosomal protein S6, SMA: smooth muscle actin, STAT3: Signal transducer and activator of transcription 3, TIMP-2: tissue inhibitor of metalloproteinase 2, TK1: thymidine kinase 1, TOP2A: topoisomerase 2-alpha, VEGF: vascular endothelial growth factor

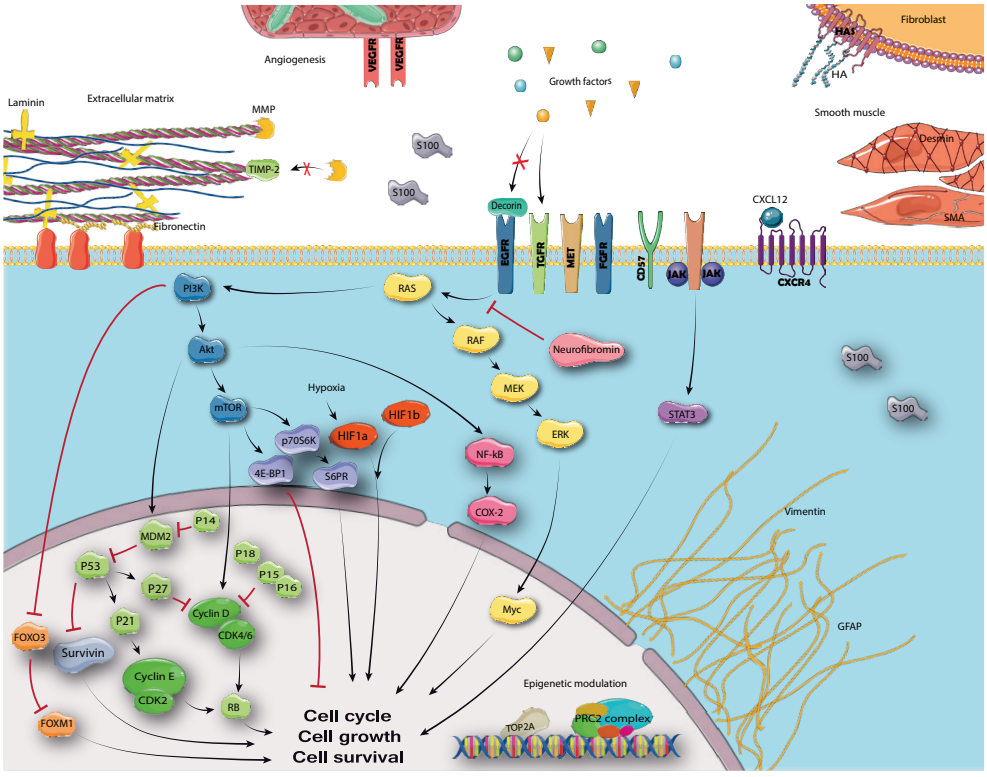

Figure 2. Cellular pathways in MPNST

Differentiation

Seven mesenchymal and neuronal differentiation markers were evaluated (**Table 2**), most commonly S100. (4, 21-25) In univariable analysis complete absence of S100 was found negatively associated with survival in 4/6 studies. Two studies showed the absence of S100 to be an independent predictor of worse survival with HR 4.5 (95% CI: 2.0-12.1) and HR 6.6 (95% CI: 1.8-23.8). (4, 21) All seven markers were also evaluated for association with disease-free survival (DFS). Negative S100 staining was associated with worse DFS in 2/4 studies, of which one study showed an independent association (HR 4.2, 95% CI: 1.5-12.3). (21) Negative smooth muscle actin (SMA) and CD57 staining were also found associated with worse DFS in univariable analysis in one study, but not in multivariable analysis. (22)

Table 2. Prognostic value of immunohistochemical markers

Marker	N	Survival[a]				N	Disease-free survival[a]			
		Univariable	Multivariable				Univariable	Multivariable		
		+	*NA*	+	-		+	*NA*	+	-
Differentiation										
S100	7	57%	25%	50%	25%	4	50%	50%	50%	0%
GFAP	2	0%	*NA*	*NA*	*NA*	2	0%	*NA*	*NA*	*NA*
Vimentin	2	0%	*NA*	*NA*	*NA*	2	0%	*NA*	*NA*	*NA*
NSE	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
SMA	1	0%	*NA*	*NA*	*NA*	1	100%	0%	0%	100%
Desmin	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
CD57	1	0%	*NA*	*NA*	*NA*	1	100%	0%	0%	100%
Vascularization										
MVD	4	25%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
VEGF	2	50%	100%	0%	0%	0	*NA*	*NA*	*NA*	*NA*
Receptors and ligands										
EGFR	3	67%	100%	0%	0%	1	100%	100%	0%	0%
MET	2	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
p-MET	1	100%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
HGF	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
CXCR4	1	100%	0%	0%	100%	1	0%	*NA*	*NA*	*NA*
CXCL12	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
FGFR1	1	100%	0%	100%	0%	1	0%	*NA*	*NA*	*NA*
FGFR2	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
FGFR4	1	0%	*NA*	*NA*	*NA*	1	100%	0%	0%	100%
HA	1	100%	0%	0%	100%	1	100%	0%	100%	0%
HAS1	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
HAS2	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
HAS3	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
Decorin	1	100%	100%	0%	0%	0	*NA*	*NA*	*NA*	*NA*
Ras pathway										
p-MEK	2	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
NF	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
p-ERK	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
p-Akt	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
p-mTOR	1	100%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
p-p70S6K	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
p-4E-BP1	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
p-S6RP	1	100%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
COX-2	1	100%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
Myc	1	100%	0%	0%	100%	0	*NA*	*NA*	*NA*	*NA*
Cell cycle regulation										
p53	10	40%	25%	75%	0%	3	67%	50%	50%	0%
MDM2	4	0%	*NA*	*NA*	*NA*	2	50%	100%	0%	0%
Cyclin D1	4	25%	0%	100%	0%	3	33%	0%	100%	0%
p21	3	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*

Marker	N	Survival[a]				N	Disease-free survival[a]			
		Univariable	Multivariable				Univariable	Multivariable		
		+	*NA*	+	-		+	*NA*	+	-
Cyclin E	2	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
p-RB	2	0%	*NA*	*NA*	*NA*	1	100%	100%	0%	0%
p14	2	100%	0%	50%	50%	0	*NA*	*NA*	*NA*	*NA*
p16	2	50%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
p27	2	50%	100%	0%	0%	1	0%	*NA*	*NA*	*NA*
p15	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
p18	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
FOXM1	1	100%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
SOX5	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
CDK2	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
CDK4	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
Cyclin D3	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
HIF1α	1	100%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
HIF2α	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
CHFR	1	100%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
Epigenetic modulation										
H3K27me3	3	67%	0%	50%	50%	1	0%	*NA*	*NA*	*NA*
TOP2A	2	100%	50%	0%	50%	1	100%	100%	0%	0%
Other										
Ki67	13	62%	0%	25%	75%	5	40%	0%	50%	50%
Survivin	2	50%	0%	0%	100%	1	0%	*NA*	*NA*	*NA*
ATRX	1	100%	0%	100%	0%	0	*NA*	*NA*	*NA*	*NA*
TK1	1	100%	0%	0%	100%	0	*NA*	*NA*	*NA*	*NA*
MCD	1	0%	*NA*	*NA*	*NA*	0	*NA*	*NA*	*NA*	*NA*
p-STAT3	1	100%	100%	0%	0%	1	0%	*NA*	*NA*	*NA*
Osteopontin	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
Fibronectin	1	0%	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
MMP-2	0	*NA*	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
MMP-9	0	*NA*	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
MMP-13	0	*NA*	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
TIMP-2	0	*NA*	*NA*	*NA*	*NA*	1	0%	*NA*	*NA*	*NA*
Laminin receptor	0	*NA*	*NA*	*NA*	*NA*	1	100%	100%	*NA*	*NA*

[a]Univariable analysis: significant effect (+), not significant effect (-); Multivariable analysis: not performed (*NA*), significant effect (+), nog significant effect (-)

4E-BP1: eukaryotic translation initiation factor 4E-binding protein 1, CDK: cyclin dependent kinase, CHFR: checkpoint with forkhead-associated domain and ring finger, COX-2: cyclooxygenase-2, CXCR4: C-X-C motif chemokine receptor 4, CXCL12: C-X-C motif chemokine ligand 12, DFS: disease-free survival (either time to recurrence, metastasis, or both), EGFR: epidermal growth factor receptor, ERK: extracellular signal-regulated kianses, FGFR: fibroblast growth factor receptor, FOXM1: forkhead box protein M1, GFAP: glial fibrillary acidic protein, H3K27me3: trimethylation of lysine 27 of histone H3, HA: hyaluronan, HAS: hyaluronan synthase, HIF: hypoxia-inducible factor, MCD: mast cell density, MDM2: mouse double minute 2 homolog, MEK: mitogen-activated protein kinase kinase, MMP: matrix metalloproteinase, mTOR: mammalian target of rapamycin, MVD: microvessel density, N: number of studies, NA: not applicable, NF: neurofibromin, p-: phosphorylated, S: survival (either disease-specific or overall), S6RP: ribosomal protein S6, SMA: smooth muscle actin, STAT3: Signal transducer and activator of transcription 3, TIMP-2: tissue inhibitor of metalloproteinase 2, TK1: thymidine kinase 1, TOP2A: topoisomerase 2-alpha, VEGF: vascular endothelial growth factor

Vascularization

Microvascular densitiy (MVD) and vascular epithelial growth factor (VEGF) staining were evaluated as vascularization markers (**Table 2**). (4, 26-29) High MVD was associated with worse survival in 1/4 studies. This association was also significant in multivariable analyses (HR 7.3, 95% CI: 1.4-38.5). (29) High VEGF staining was associated with worse survival in 1/2 studies, but this was not studied in a multivariable model. (26) No markers were studied for association with DFS.

Receptors and ligands

Immunohistochemical expression of 9 different receptors or their ligands were evaluated, most commonly the epidermal growth factor receptor (EGFR, **Table 2**). (4, 23, 30-36) Increased EGFR staining was associated with worse survival in univariable analysis in 2/3 studies, but this was not evaluated in a multivariable model. (4, 30, 32) Increased phosphorylated MET (p-MET), C-X-C motif chemokine receptor 4 (CXCR4), and low fibroblast growth factor receptor 1 (FGFR1) staining were also associated with worse survival in univariable analysis, but only p-MET (HR 1.04, 95% CI: 1.0-1.1) and FGFR1 (HR 2.8, 95% CI: 1.2-6.7) were independently associated with survival. (23, 31, 34, 36) Increased EGFR and FGFR4 were associated with worse DFS, but only in univariable analyses. (30, 36) On a genetic level, no amplification of *FGFR1* on fluorescence in situ hybridization (FISH) was associated with worse survival and DFS in univariable analysis (**Appendix B**). (36) Copy number alterations in *ERBB2* were not associated with survival. (37)

Extracellular matrix

Twelve extracellular matrix markers were studied, of which none was evaluated more than once (**Table 2**). (34, 35, 38-41) Only increased hyaluronan (HA) and decorin staining were associated with decreased survival, but none in a multivariable model. (34, 35) Increased HA and laminin receptor were associated with worse DFS, but only HA was associated with worse DFS in a multivariable model (HR 5.7, 95% CI: 1.2-26.4). (34, 38)

Ras pathway

Ten different Ras pathway proteins were stained, but only phosphorylated MAPK kinase (MEK) was evaluated more than once (**Table 2**). (4, 7, 21, 37, 42) Increased phosphorylated mammalian target of rapamycin (p-mTOR), phosphorylated ribosomal protein S6 (p-S6RP), cyclooxygenase-2 (COX-2), and Myc staining were associated with worse survival univariable analysis. (7, 37, 42) Only increased p-mTOR (HR 2.6, 95% CI: 1.3-5.5), p-S6RP (HR 2.5, 95% CI: 1.3-5.5), and COX-2 (HR 3.0, 95% CI: 1.1-10.2) staining were independently associated with worse survival. (7, 42) No Ras pathway associated immunohistochemical marker was found associated with

DFS. On a genetic level, copy number alterations of *MYC* were not associated with survival. (37) Methylation of *RASSF1A* gene was associated independently with worse survival in one study (HR 5.2, 95% CI: 1.4-19.4, **Appendix B**). (43) This association was however only found in the NF1 subpopulation.

Cell cycle regulation

Sixteen immunohistochemical markers of cell cycle regulation were evaluated, most commonly p53 (**Table 2**). (4, 23, 24, 27, 31, 33, 37, 40, 44-52) Low p14, p16, checkpoint with forkhead-associated domain and ring finger (CHFR), and increase in p53, p14, cyclin D1, p27, and forkhead box protein M1 (FOXM1) staining were associated with worse survival in univariable analysis. (4, 23, 27, 37, 40, 44, 45, 48, 51) Positive p53 staining was independently associated with survival in 3/4 studies (HR 1.8, 95% CI: 1.0-3.3, HR 2.3, 95% CI: 1.2-4.5, and HR 6.4, 95% CI: 1.5-29.0). (4, 23, 52) Increased staining of cyclin D1 (HR 15.9, 95% CI: 2.0-125.0), HIF1α (HR 8.3, 95% CI: 2.8-28.9), FOXM1 (HR 1.9, 95% CI: 1.1-3.3), and decreased staining of p16 (HR 2.2, 95% CI: 1.5-3.2) and p14 (HR 2.7, 95% CI: 1.8-4.2) were also independently associated with worse survival in one study each. (27, 28, 37, 40) Positive staining of p53, MDM2, cyclin D1, and p-RB were associated with worse DFS in univariable analysis. (33, 40, 48) Only cyclin D1 (HR 11.1, 95% CI: 2.8-47.6) and p53 (HR 3.2, 95% CI: 1.0-10.4) were independently associated with worse DFS in one study. (40) On a genetic level, mutation, homozygous loss, or loss of heterogeneity of *TP53* was associated with worse survival in 2/3 studies (**Appendix B**). (37, 41, 53) The copy number gain of *MDM2* and *CDK4* as well as amplification on FISH of *CDK4* were associated with worse survival. (37, 53) Gain (HR 4.2, 95% CI: 1.4-12.4) or amplification (HR 2.0, 95% CI: 1.0-4.0) of *CDK4* was independently associated with worse survival. (37) The combination of either *MDM2* gain or *TP53* aberration made a high-risk group (16%) for worse survival with a HR 3.4 (95% CI: 1.4-8.3). (53) In the same study, a gene expression profile was made and a score of ≥0.12 was present in 66.7% of the population which was associated with worse survival as well (HR 4.0, 95% CI: 1.3-12.1). Another study on DNA copy number changes found a significant association with worse survival for gain at 17q23.2-25.3, but not in several related genes or micro-RNAs in this region. (54) The association was not evaluated in a multivariable model. A gain in *FOXM1* was worse survival in another study. (37) Only the polymorphism of $p53Pro^{72}$ was associated with worse DFS in one study. (41) This association was not evaluated in a multivariable model.

Epigenetic modulation

Two epigenetic modulating proteins were investigated as immunohistochemical markers (**Table 2**). (44-46, 55, 56) Loss of trimethylation of lysine 27 of histone

H3 (H3K27me3) and increased topoisomerase 2-alpha (TOP2A) staining were both associated with decreased survival. (44, 45, 55, 56) Only H3K27me3 was independently associated with worse survival (HR 2.6, 95% CI: 1.2-5.7) in one out of two studies. (44, 45) Increased TOP2A staining was also associated with worse DFS in one study. (55) High copy number changes of *TOP2A* were not associated with worse survival (**Appendix B**). (54)

Other

Thirteen other immunohistochemical markers were studied, most commonly the proliferation marker Ki67. (4, 7, 21, 22, 28, 29, 37, 40, 47, 48, 51, 52, 55-59) On average a cut-off at 20.9% (range: 5-30%) for high Ki67 staining was used and it was significantly associated with worse survival in 8/12 studies, of which two studies showed an independent association (HR 2.4, 95% CI: 1.1-4.9 and HR 10.2, 95% CI: 3.6-32.1). (27, 28) Increased survivin, thymidine kinase 1 (TK1), phosphorylated signal transducer and activator of transcription 3 (p-STAT3), and hypoxia-induced factor 1-alpha (HIF1α) and decreased ATRX staining were associated with worse survival. (28, 56, 57) Both decreased ATRX (HR 5.3, 95% CI: 1.4-20.4) and positive HIF1α staining (HR 8.3, 95% CI: 2.8-28.9) were independently associated with worse survival. (28, 57) One study showed that when there was high survivin and high TK1 staining or low survivin and high TOP2A staining a high-risk group of patients could be stratified with HR 4.6 (95% CI: 1.5-14.4). (56) Increased staining of Ki67 and laminin receptor were associated with worse DFS. (21, 38, 58) Only high Ki67 staining was shown to have an independent association with worse DFS in 1/2 studies (HR 3.8, 95% CI: 1.7-8.5). (21) Four studies investigated several other genetic alterations, including two on *BIRC5*, the gene encoding survivin. (54, 60-62) One out of two studies showed that an increase in *BIRC5* mRNA was associated with worse survival in univariable analysis. (60) Gain at 17q23.2-25.3 was associated with worse survival in univariable analysis in another study. (54) One study investigated the effect of chromosomal gains and losses and showed an independent effect on worse survival for Xq loss (HR 3.6, 95% CI: 1.6-8.3), 10q loss (HR 3.2, 95% CI: 1.4-7.7), and 16p gain (HR 2.5, 95% CI: 1.0-6.2). (62) Together a high-risk group (63% of population) was obtained for either gain or loss which resulted in a HR 11.0 (95% CI: 3.5-35.0) after correction for several clinical characteristics. A gain in *SOX5* and *NOL1* were associated with worse survival in one study, but only in univariable analyses. (37) Finally, methylation of *MAGEA3* was also associated with worse survival in univariable analysis. (61)

Discussion

The underlying biology of MPNSTs remains complex as is highlighted by the diverse findings of studies included in this review. Many markers and genetic alterations have been proposed to be of prognostic value, yet outcomes are infrequently repeated. Alterations in *TP53* or its resulting increased p53 staining were commonly found associated with survival and DFS as were several other proteins and genes involved in cell cycle regulation. Epigenetic modulatory proteins, especially loss of H3K27me3, and more general markers as absence of S100 and increased Ki67 were commonly found to be of prognostic value too.

Prognostication in MPNST

The predictive value of clinical parameters including patient and tumour characteristics has been studied more commonly than immunohistochemical or genetic biomarkers in MPNST. Increasing age, large tumour size, metastatic disease at diagnosis, and tumours not amenable to complete resection are the most commonly found predictors of worse survival in MPNST. (2, 3, 5, 13, 25, 63) This emphasizes the importance of early diagnosis of MPNST in order to completely resect tumours, along with finding new systemic therapies to improve the prognosis of irresectable and metastatic disease. Non-extremity tumour sites have also been shown to have a negative impact on survival, however this may be truer for those arising in retroperitoneal or pelvic sites. (1, 3, 5, 13, 64) Tumour depth used to be incorporated for prognostication in the AJCC staging system for STS but has varyingly been shown to be of prognostic value in MPNST. (2, 3, 5, 13, 25, 63) The importance of NF1 disease has also been subject of debate. A meta-analysis in 2012 showed no difference in survival for patients in papers published after 2000. (15) However, recent large cohorts did find an independent association with worse survival for NF1 patients. (3, 13, 65, 66) Altogether, clinical parameters seem to be able to predict some part of a patient's course of disease. The addition of tumour biology to clinical parameters may further increase our ability to stratify subgroups of patients based on prognosis. *TP53* is one of the few recurrently mutated genes found in MPNST. *TP53* mutations and high p53 staining were independently associated with survival or DFS in 5 different studies. (4, 23, 40, 52, 53) This may indicate that aberrations in this gene may indeed be of clinical importance. Other genes involved in cell cycle regulation such as *CDKN2A* and downstream proteins are commonly altered and may not only contribute to tumourigenesis but also be of clinical significance, supporting a belief that dysregulations in this cellular pathway are of overall importance. Loss of polycomb regressive complex 2 (PRC2) has recently been shown to be common in MPNSTs due to mutations in *EED* and *SUZ12*. (9, 67) This results in loss of H3K27me3 which can reliably distinguish high-grade MPNSTs from their benign counterparts by immunohistochemistry. (68, 69) MPNSTs without

loss of H3K27me3 staining may also be associated with less aggressive behaviour as many low-grade MPNSTs are known to retain this expression. (14, 44) Preclinical research on targeted therapies has most frequently shown promising results targeting proteins in the Ras pathway, especially when combined with other target drugs, but unfortunately no clinical trial has proven benefit to date. (20) Activated proteins in the Ras pathway, including p-mTOR, p-4E-BP1, p-S6RP, COX-2, and Myc as well as methylation of *RASSF1A* may however predict worse survival. (7, 37, 42, 43) Targeting vascular pathways in MPNSTs may be beneficial, but unfortunately few studies have focused on this. Studies included in this review also showed that increased vascularity, as evidenced by increased microvascular density as well as increased expression of VEGF, may be associated with more aggressive biological behaviour. (26, 29) It seems that many other targets may be of prognostic value as well emphasizing the need for further research into MPNST tumour biology. Survivin markers may for instance stratify a subgroup of patients and survivin has been shown a viable target in a xenograft mouse model. (70) Seeing as MPNSTs are heterogenic and markers such as p53 are not MPNST specific, combined scores of different markers and genetic alterations may be of most clinical importance. Four studies in this review highlight this phenomenon demonstrating increased prognostic value when markers are combined. (27, 53, 56, 62)

Strengths and limitations

Unfortunately, due to the large heterogeneity of published studies meta-analyses were not presumed feasible. All studies included in this review were retrospective of nature inherently harbouring bias. None of the markers and genetic alterations found in these studies were prospectively validated. Moreover, many did not evaluate the prognostic value of their markers in a multivariable model nor on their discriminative ability. Studies that evaluated the prognostic value of markers in a multivariable model were nonetheless not always capable to correct for all common clinical variables. MPNSTs are rare sarcomas, which in combination with their complex biology, makes it difficult to obtain enough cases to create valuable models. But as shown in this review, several markers and genetic alterations may already be of clinical importance as they have shown an independent association with survival in addition to clinical parameters. Future research should therefore be encouraged to replicate these results using larger datasets obtained by large-scale international collaborations. Important immunohistochemical staining may include Ki67, S100, p53, and H3K27me3 in all patients, and possibly further staining of proteins associated with cell cycle regulation. In turn individual prediction models for MPNST patients specifically may arise taking their significant heterogeneity into account. Such models may better elucidate patient selection for (neo)adjuvant treatment and targeted therapies, which should then be validated in a prospective database. But as MPNSTs remain rare entities one may also turn to exploratory analyses

using machine learning techniques on large STS genetic databases to identify attractive genes as biomarkers or prognostic markers in subtypes of STS. (71)

Conclusion

MPNSTs harbour complex and heterogenic biology and currently lack adequate staging systems. Immunohistochemical markers and genetic alterations are varyingly of prognostic value. Absence of S100 and H3K27me3 and increased Ki67 staining were commonly found to be of independent prognostic value alongside of clinical parameters. Alterations in *TP53* or its consequential increase in p53 staining seems to distinguish a subgroup of MPNSTs with worse outcomes. Immunohistochemical staining and associated genetic alterations of proteins involved in cell cycle regulation and the Ras pathway may also help stratifying patients with worse outcomes. Ideal staining of these pathways for prognostic purposes has yet to be determined. Other markers will likely need further evaluation for validation. A combination of markers may increase the prognostic value.

References

1. Stucky C-CH, Johnson KN, Gray RJ, Pockaj BA, Ocal IT, Rose PS, et al. Malignant peripheral nerve sheath tumors (MPNST): the Mayo Clinic experience. Annals of surgical oncology. 2012;19(3):878-85.
2. Valentin T, Le Cesne A, Ray-Coquard I, Italiano A, Decanter G, Bompas E, et al. Management and prognosis of malignant peripheral nerve sheath tumors: The experience of the French Sarcoma Group (GSF-GETO). European journal of cancer (Oxford, England : 1990). 2016;56:77-84.
3. Martin E, Coert JH, Flucke UE, Slooff WBM, Ho VKY, van der Graaf WT, et al. A nationwide cohort study on treatment and survival in patients with malignant peripheral nerve sheath tumours. European Journal of Cancer. 2020;124:77-87.
4. Zou C, Smith KD, Liu J, Lahat G, Myers S, Wang W-L, et al. Clinical, pathological, and molecular variables predictive of malignant peripheral nerve sheath tumor outcome. Ann Surg. 2009;249(6):1014-22.
5. Anghileri M, Miceli R, Fiore M, Mariani L, Ferrari A, Mussi C, et al. Malignant peripheral nerve sheath tumors: prognostic factors and survival in a series of patients treated at a single institution. Cancer. 2006;107(5):1065-74.
6. Basu TN, Gutmann DH, Fletcher JA, Glover TW, Collins FS, Downward J. Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. Nature. 1992;356(6371):713-5.
7. Endo M, Yamamoto H, Setsu N, Kohashi K, Takahashi Y, Ishii T, et al. Prognostic significance of AKT/mTOR and MAPK pathways and antitumor effect of mTOR inhibitor in NF1-related and sporadic malignant peripheral nerve sheath tumors. Clin Cancer Res. 2013;19(2):450-61.
8. Beert E, Brems H, Daniëls B, De Wever I, Van Calenbergh F, Schoenaers J, et al. Atypical neurofibromas in neurofibromatosis type 1 are premalignant tumors. Genes Chromosomes Cancer. 2011;50(12):1021-32.
9. De Raedt T, Beert E, Pasmant E, Luscan A, Brems H, Ortonne N, et al. PRC2 loss amplifies Ras-driven transcription and confers sensitivity to BRD4-based therapies. Nature. 2014;514(7521):247-51.
10. Legius E, Dierick H, Wu R, Hall BK, Marynen P, Cassiman JJ, et al. TP53 mutations are frequent in malignant NF1 tumors. Genes Chromosomes Cancer. 1994;10(4):250-5.
11. Masliah-Planchon J, Pasmant E, Luscan A, Laurendeau I, Ortonne N, Hivelin M, et al. MicroRNAome profiling in benign and malignant neurofibromatosis type 1-associated nerve sheath tumors: evidences of PTEN pathway alterations in early NF1 tumorigenesis. BMC Genomics. 2013;14:473-.
12. Kluwe L, Friedrich RE, Mautner VF. Allelic loss of the NF1 gene in NF1-associated plexiform neurofibromas. Cancer Genet Cytogenet. 1999;113(1):65-9.
13. Miao R, Wang H, Jacobson A, Lietz AP, Choy E, Raskin KA, et al. Radiation-induced and neurofibromatosis-associated malignant peripheral nerve sheath tumors (MPNST) have worse outcomes than sporadic MPNST. Radiother Oncol. 2019;137:61-70.
14. Miettinen MM, Antonescu CR, Fletcher CDM, Kim A, Lazar AJ, Quezado MM, et al. Histopathologic evaluation of atypical neurofibromatous tumors and their transformation into malignant peripheral nerve sheath tumor in patients with neurofibromatosis 1-a consensus overview. Hum Pathol. 2017;67:1-10.
15. Kolberg M, Høland M, Agesen TH, Brekke HR, Liestøl K, Hall KS, et al. Survival meta-analyses for >1800 malignant peripheral nerve sheath tumor patients with and without neurofibromatosis type 1. Neuro Oncol. 2013;15(2):135-47.

16. Gronchi A, Ferrari S, Quagliuolo V, Broto JM, Pousa AL, Grignani G, et al. Histotype-tailored neoadjuvant chemotherapy versus standard chemotherapy in patients with high-risk soft-tissue sarcomas (ISG-STS 1001): an international, open-label, randomised, controlled, phase 3, multicentre trial. The Lancet Oncology. 2017;18(6):812-22.
17. Higham CS, Steinberg SM, Dombi E, Perry A, Helman LJ, Schuetze SM, et al. SARC006: Phase II Trial of Chemotherapy in Sporadic and Neurofibromatosis Type 1 Associated Chemotherapy-Naive Malignant Peripheral Nerve Sheath Tumors. Sarcoma. 2017;2017:8685638.
18. Carli M, Ferrari A, Mattke A, Zanetti I, Casanova M, Bisogno G, et al. Pediatric malignant peripheral nerve sheath tumor: the Italian and German soft tissue sarcoma cooperative group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(33):8422-30.
19. Zehou O, Fabre E, Zelek L, Sbidian E, Ortonne N, Banu E, et al. Chemotherapy for the treatment of malignant peripheral nerve sheath tumors in neurofibromatosis 1: a 10-year institutional review. Orphanet J Rare Dis. 2013;8:127-.
20. Martin E, Lamba N, Flucke UE, Verhoef C, Coert JH, Versleijen-Jonkers YMH, et al. Non-cytotoxic systemic treatment in malignant peripheral nerve sheath tumors (MPNST): A systematic review from bench to bedside. Crit Rev Oncol Hematol. 2019;138:223-32.
21. Yuan Z, Xu L, Zhao Z, Xu S, Zhang X, Liu T, et al. Clinicopathological features and prognosis of malignant peripheral nerve sheath tumor: a retrospective study of 159 cases from 1999 to 2016. Oncotarget. 2017;8(62):104785-95.
22. Wang T, Yin H, Han S, Yang X, Wang J, Huang Q, et al. Malignant peripheral nerve sheath tumor (MPNST) in the spine: a retrospective analysis of clinical and molecular prognostic factors. J Neurooncol. 2015;122(2):349-55.
23. Torres KE, Zhu Q-S, Bill K, Lopez G, Ghadimi MP, Xie X, et al. Activated MET is a molecular prognosticator and potential therapeutic target for malignant peripheral nerve sheath tumors. Clin Cancer Res. 2011;17(12):3943-55.
24. Le Guellec S, Decouvelaere A-V, Filleron T, Valo I, Charon-Barra C, Robin Y-M, et al. Malignant Peripheral Nerve Sheath Tumor Is a Challenging Diagnosis: A Systematic Pathology Review, Immunohistochemistry, and Molecular Analysis in 160 Patients From the French Sarcoma Group Database. Am J Surg Pathol. 2016;40(7):896-908.
25. LaFemina J, Qin L-X, Moraco NH, Antonescu CR, Fields RC, Crago AM, et al. Oncologic outcomes of sporadic, neurofibromatosis-associated, and radiation-induced malignant peripheral nerve sheath tumors. Annals of surgical oncology. 2013;20(1):66-72.
26. Wasa J, Nishida Y, Suzuki Y, Tsukushi S, Shido Y, Hosono K, et al. Differential expression of angiogenic factors in peripheral nerve sheath tumors. Clin Exp Metastasis. 2008;25(7):819-25.
27. Endo M, Kobayashi C, Setsu N, Takahashi Y, Kohashi K, Yamamoto H, et al. Prognostic significance of p14ARF, p15INK4b, and p16INK4a inactivation in malignant peripheral nerve sheath tumors. Clin Cancer Res. 2011;17(11):3771-82.
28. Fukushima S, Endo M, Matsumoto Y, Fukushi JI, Matsunobu T, Kawaguchi KI, et al. Potential therapeutic target in malignant peripheral nerve sheath tumor. PLoS One. 2017;12(5).
29. Vasconcelos RATd, Guimarães Coscarelli P, Vieira TM, Noguera WS, Rapozo DCM, Acioly MA. Prognostic significance of mast cell and microvascular densities in malignant peripheral nerve sheath tumor with and without neurofibromatosis type 1. Cancer Med. 2019;8(3):972-81.
30. Keizman D, Issakov J, Meller I, Maimon N, Ish-Shalom M, Sher O, et al. Expression and significance of EGFR in malignant peripheral nerve sheath tumor. J Neurooncol. 2009;94(3):383-8.

31. Zhang C, Chang F-Y, Zhou W-Y, Yang J-L. The prognostic value of C-X-C motif chemokine receptor 4 in patients with sporadic malignant peripheral nerve sheath tumors. Chin J Cancer. 2017;36(1):80-.
32. Tabone-Eglinger S, Bahleda R, Côté J-F, Terrier P, Vidaud D, Cayre A, et al. Frequent EGFR Positivity and Overexpression in High-Grade Areas of Human MPNSTs. Sarcoma. 2008;2008:849156-.
33. Fan Q, Yang J, Wang G. Clinical and molecular prognostic predictors of malignant peripheral nerve sheath tumor. Clin Transl Oncol. 2014;16(2):191-9.
34. Ikuta K, Urakawa H, Kozawa E, Arai E, Zhuo L, Futamura N, et al. Hyaluronan expression as a significant prognostic factor in patients with malignant peripheral nerve sheath tumors. Clin Exp Metastasis. 2014;31(6):715-25.
35. Jia X, Chen C, Chen L, Yu C, Kondo T. Decorin as a prognostic biomarker in patients with malignant peripheral nerve sheath tumors. Oncol Lett. 2019;17(3):3517-22.
36. Zhou W, Du X, Song F, Zheng H, Chen K, Zhang W, et al. Prognostic roles for fibroblast growth factor receptor family members in malignant peripheral nerve sheath tumor. Oncotarget. 2016;7(16):22234-44.
37. Yu J, Deshmukh H, Payton JE, Dunham C, Scheithauer BW, Tihan T, et al. Array-based comparative genomic hybridization identifies CDK4 and FOXM1 alterations as independent predictors of survival in malignant peripheral nerve sheath tumor. Clin Cancer Res. 2011;17(7):1924-34.
38. Benassi MS, Ragazzini P, Gamberi G, Sollazzo MR, Molendini L, Ferrari C, et al. Adhesion molecules in high-grade soft tissue sarcomas: correlation to clinical outcome. European journal of cancer (Oxford, England : 1990). 1998;34(4):496-502.
39. Benassi MS, Gamberi G, Magagnoli G, Molendini L, Ragazzini P, Merli M, et al. Metalloproteinase expression and prognosis in soft tissue sarcomas. Annals of oncology : official journal of the European Society for Medical Oncology. 2001;12(1):75-80.
40. Krawczyk MA, Karpinsky G, Izycka-Swieszewska E, Gabrych A, Kunc M, Fatyga A, et al. Immunohistochemical assessment of cyclin D1 and p53 is associated with survival in childhood malignant peripheral nerve sheath tumor. Cancer Biomark. 2019;24(3):351-61.
41. Holtkamp N, Atallah I, Okuducu A-F, Mucha J, Hartmann C, Mautner V-F, et al. MMP-13 and p53 in the progression of malignant peripheral nerve sheath tumors. Neoplasia. 2007;9(8):671-7.
42. Hakozaki M, Tajino T, Konno S, Kikuchi S, Yamada H, Yanagisawa M, et al. Overexpression of cyclooxygenase-2 in malignant peripheral nerve sheath tumor and selective cyclooxygenase-2 inhibitor-induced apoptosis by activating caspases in human malignant peripheral nerve sheath tumor cells. PLoS One. 2014;9(2):e88035-e.
43. Danielsen SA, Lind GE, Kolberg M, Høland M, Bjerkehagen B, Hall KS, et al. Methylated RASSF1A in malignant peripheral nerve sheath tumors identifies neurofibromatosis type 1 patients with inferior prognosis. Neuro Oncol. 2015;17(1):63-9.
44. Cleven AHG, Al Sannaa GA, Briaire-de Bruijn I, Ingram DR, Van De Rijn M, Rubin BP, et al. Loss of H3K27 tri-methylation is a diagnostic marker for malignant peripheral nerve sheath tumors and an indicator for an inferior survival. Modern Pathology. 2016;29(6):582-90.
45. Otsuka H, Kohashi K, Yoshimoto M, Ishihara S, Toda Y, Yamada Y, et al. Immunohistochemical evaluation of H3K27 trimethylation in malignant peripheral nerve sheath tumors. Pathol Res Pract. 2018;214(3):417-25.
46. Pekmezci M, Cuevas-Ocampo AK, Perry A, Horvai AE. Significance of H3K27me3 loss in the diagnosis of malignant peripheral nerve sheath tumors. Mod Pathol. 2017;30(12):1710-9.
47. Watanabe T, Oda Y, Tamiya S, Kinukawa N, Masuda K, Tsuneyoshi M. Malignant peripheral nerve sheath tumours: high Ki67 labelling index is the significant prognostic indicator. Histopathology. 2001;39(2):187-97.

48. Kourea HP, Cordon-Cardo C, Dudas M, Leung D, Woodruff JM. Expression of p27(kip) and other cell cycle regulators in malignant peripheral nerve sheath tumors and neurofibromas: the emerging role of p27(kip) in malignant transformation of neurofibromas. Am J Pathol. 1999;155(6):1885-91.
49. Leroy K, Dumas V, Martin-Garcia N, Falzone MC, Voisin MC, Wechsler J, et al. Malignant peripheral nerve sheath tumors associated with neurofibromatosis type 1: a clinicopathologic and molecular study of 17 patients. Arch Dermatol. 2001;137(7):908-13.
50. Halling KC, Scheithauer BW, Halling AC, Nascimento AG, Ziesmer SC, Roche PC, et al. p53 expression in neurofibroma and malignant peripheral nerve sheath tumor. An immunohistochemical study of sporadic and NF1-associated tumors. Am J Clin Pathol. 1996;106(3):282-8.
51. Kobayashi C, Oda Y, Takahira T, Izumi T, Kawaguchi K, Yamamoto H, et al. Aberrant expression of CHFR in malignant peripheral nerve sheath tumors. Mod Pathol. 2006;19(4):524-32.
52. Brekke HR, Kolberg M, Skotheim RI, Hall KS, Bjerkehagen B, Risberg B, et al. Identification of p53 as a strong predictor of survival for patients with malignant peripheral nerve sheath tumors. Neuro Oncol. 2009;11(5):514-28.
53. Høland M, Kolberg M, Danielsen SA, Bjerkehagen B, Eilertsen IA, Hektoen M, et al. Inferior survival for patients with malignant peripheral nerve sheath tumors defined by aberrant TP53. Mod Pathol. 2018;31(11):1694-707.
54. Kresse SH, Skårn M, Ohnstad HO, Namløs HM, Bjerkehagen B, Myklebost O, et al. DNA copy number changes in high-grade malignant peripheral nerve sheath tumors by array CGH. Mol Cancer. 2008;7:48-.
55. Skotheim RI, Kallioniemi A, Bjerkhagen B, Mertens F, Brekke HR, Monni O, et al. Topoisomerase-II alpha is upregulated in malignant peripheral nerve sheath tumors and associated with clinical outcome. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21(24):4586-91.
56. Kolberg M, Høland M, Lind GE, Ågesen TH, Skotheim RI, Hall KS, et al. Protein expression of BIRC5, TK1, and TOP2A in malignant peripheral nerve sheath tumours--A prognostic test after surgical resection. Mol Oncol. 2015;9(6):1129-39.
57. Lu H-C, Eulo V, Apicelli AJ, Pekmezci M, Tao Y, Luo J, et al. Aberrant ATRX protein expression is associated with poor overall survival in NF1-MPNST. Oncotarget. 2018;9(33):23018-28.
58. Gong H, Zhang D, Wang D, He S, Yang X, Wei H, et al. Benign-appearing Intraspinal Malignant Peripheral Nerve Sheath Tumors: Treatments and Outcomes of 14 Consecutive Patients. Turk Neurosurg. 2018;28(6):983-8.
59. Panse G, Leung CH, Ingram DR, Wani K, Torres KE, Lin H, et al. The role of phosphorylated signal transducer and activator of transcription 3 (pSTAT3) in peripheral nerve sheath tumours. Histopathology. 2017;70(6):946-53.
60. Alaggio R, Turrini R, Boldrin D, Merlo A, Gambini C, Ferrari A, et al. Survivin expression and prognostic significance in pediatric malignant peripheral nerve sheath tumors (MPNST). PLoS One. 2013;8(11):e80456-e.
61. Nobeyama Y, Nakagawa H. MAGEA3 methylation status is associated with prognosis of malignant peripheral nerve sheath tumor and with neurofibroma type in neurofibromatosis type 1. J Dermatol Sci. 2016;84(1):101-4.
62. Brekke HR, Ribeiro FR, Kolberg M, Agesen TH, Lind GE, Eknaes M, et al. Genomic changes in chromosomes 10, 16, and X in malignant peripheral nerve sheath tumors identify a high-risk patient group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(9):1573-82.

63. Watson KL, Al Sannaa GA, Kivlin CM, Ingram DR, Landers SM, Roland CL, et al. Patterns of recurrence and survival in sporadic, neurofibromatosis Type 1-associated, and radiation-associated malignant peripheral nerve sheath tumors. J Neurosurg. 2017;126(1):319-29.
64. Martin E, Muskens IS, Coert JH, Smith TR, Broekman MLD. Treatment and survival differences across tumor sites in malignant peripheral nerve sheath tumors: a SEER database analysis and review of the literature. Neurooncol Pract. 2019;6(2):134-43.
65. Martin E, Coert JH, Flucke UE, Slooff W-BM, van de Sande MAJ, van Noesel MM, et al. Neurofibromatosis-associated malignant peripheral nerve sheath tumors in children have a worse prognosis: A nationwide cohort study. Pediatric blood & cancer. 2020;67(4):e28138-e.
66. van Noesel MM, Orbach D, Brennan B, Kelsey A, Zanetti I, de Salvo GL, et al. Outcome and prognostic factors in pediatric malignant peripheral nerve sheath tumors: An analysis of the European Pediatric Soft Tissue Sarcoma Group (EpSSG) NRSTS-2005 prospective study. Pediatric blood & cancer. 2019;66(10):e27833-e.
67. Lee W, Teckie S, Wiesner T, Ran L, Prieto Granada CN, Lin M, et al. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. Nat Genet. 2014;46(11):1227-32.
68. Cleven AHG, Sannaa GAA, Briaire-de Bruijn I, Ingram DR, van de Rijn M, Rubin BP, et al. Loss of H3K27 tri-methylation is a diagnostic marker for malignant peripheral nerve sheath tumors and an indicator for an inferior survival. Mod Pathol. 2016;29(6):582-90.
69. Schaefer IM, Fletcher CD, Hornick JL. Loss of H3K27 trimethylation distinguishes malignant peripheral nerve sheath tumors from histologic mimics. Mod Pathol. 2016;29(1):4-13.
70. Ghadimi MP, Young ED, Belousov R, Zhang Y, Lopez G, Lusby K, et al. Survivin is a viable target for the treatment of malignant peripheral nerve sheath tumors. Clin Cancer Res. 2012;18(9):2545-57.
71. van Ijzendoorn DGP, Szuhai K, Briaire-de Bruijn IH, Kostine M, Kuijjer ML, Bovée JVMG. Machine learning analysis of gene expression data reveals novel diagnostic and prognostic biomarkers and identifies therapeutic targets for soft tissue sarcomas. PLoS Comput Biol. 2019;15(2):e1006826-e.

Appendix

Appendix A. Search syntaxes for the Pubmed and Embase databases

PubMed search: 10-03-2020	(((prognos*[Title/Abstract]) OR predict*[Title/Abstract]) AND ((MPNST*[Title/Abstract] OR malignant peripheral nerve sheath tum*[Title/Abstract] OR malignant neurilemmoma[Title/Abstract] OR malignant schwannoma[Title/Abstract] OR neurofibrosarcoma[Title/Abstract] OR Neurilemmoma[MeSH]))
Embase search: 10-03-2020	('prognos*':ab,ti OR 'predict*':ti,ab) AND ('MPNST*':ab,ti OR 'malignant peripheral nerve sheath tum*':ab,ti OR 'malignant neurilemmoma':ab,ti OR 'malignant schwannoma':ab,ti OR 'neurofibrosarcoma':ab,ti OR 'malignant neurilemoma'/exp) AND ([article]/lim) AND ([Embase]/lim)

Appendix B. Full data on prognostic value of markers per study

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
				Immunohistochemical markers										
Benassi, 1998 (n = 17)	Laminin receptor	>35%	35.3%	Met	+	*NA*								
Benassi, 2001 (n = 15)	MMP-2	>25%	86.7%	Met	-									
	MMP-9	>25%	26.7%	Met	-									
	TIMP-2	>10%	53.3%	Met	-									
Brekke, 2009 (n = 47)	p53	>5%	76.2%	DSS	+	HR 6.4 (1.5-29.0)	-	-	+	-	-	*NA*	*NA*	-
	p-RB	>5%	90.1%	DSS	-									
	CDK2	>5%	31.7%	DSS	-									
	CDK4	>5%	86.7%	DSS	-									
	Cyclin D1	>5%	23.2%	DSS	-[a]									
	Cyclin D3	>5%	20.8%	DSS	-									
	Cyclin E1	>5%	96.0%	DSS	-									
	p14	>5%	88.7%	DSS	+	n.s.	-	-	+	-	-	*NA*	*NA*	-
	p16	>5%	38.7%	DSS	-									
	p18	>5%	43.2%	DSS	-									
	p21	>5%	11.3%	DSS	-									
	p27	>5%	14.6%	DSS	-									
	MDM2	>5%	90.2%	DSS	-									
	Ki67	>5%	61.3%	DSS	-									
Cleven, 2016 (n = 62)	H3K27me3	Absent or low	34%	DSS	+	HR 2.6 (1.2-5.7)	+	+	*NA*	*NA*	+	*NA*	*NA*	*NA*

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
Endo, 2011 (n = 82)	p14	<50%	48.8%	OS	+	HR 2.7 (1.8-4.2)	-	-	-	+	*NA*	+	*NA*	-
	p16	<50%	50.0%	OS	+	HR 2.2 (1.5-3.2)	-	-	-	+	*NA*	+	*NA*	-
	p15	<50%	53.7%	OS	-[b]									
	Ki67	≥30%	31.8%	OS	+	n.s.	-	-	-	+	*NA*	+	*NA*	-
	MVD	≥15/HPF	50.4%	OS	-									
Endo, 2013 (n = 88)	p-Akt	>endothelium	58.0%	OS	-									
	p-mTOR	>endothelium	46.6%	OS	+	HR 2.6 (1.3-5.5)	-	-	-	-	*NA*	-	*NA*	-
	p-S6RP	>endothelium	53.4%	OS	+	HR 2.5 (1.3-5.5)	-	-	-	-	*NA*	-	*NA*	-
	p-p70S6K	>endothelium	55.7%	OS	-									
	p-4E-BP1	>endothelium	62.5%	OS	-									
	p-MEK	>endothelium	93.2%	OS	-									
	p-ERK	>endothelium	81.8%	OS	-									
	Ki67	≥30%	31.8%	OS	+	HR 2.4 (1.1-4.9)	-	-	-	-	*NA*	-	*NA*	-
Fan, 2014 (n = 56)	p53	≥50%	12.5%	OS	-									
				DFS	+	*NA*								
	MET	≥50%	19.6%	OS	-									
				DFS	-									
	MDM2	≥50%	14.3%	OS	-									
				DFS	+	*NA*								
Fukushima, 2017 (n = 82)	HIF-1α	≥10%	75.6%	OS	+	HR 8.3 (2.8-28.9)	-	-	-	-	+	-	*NA*	*NA*
	HIF-2α	Positive	34.8%	OS	-									
	MVD	>15/HPF	47.6%	OS	-									
	Ki67	≥25.8%	59.8%	OS	+	HR 10.2 (3.6-32.1)	-	-	-	-	+	-	*NA*	*NA*

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
Gong, 2018 (n = 14)	Ki67	≥20%	28.6%	OS	+	n.s.	-	-	*NA*	-	-	*NA*	-	-
				DFS	+	n.s.	-	-	*NA*	-	-	*NA*	-	-
Hakozaki, 2014 (n = 44)	COX-2	≥50%	65.9%	OS	+	HR 3.0 (1.1-10.2)	-	-	+	-	+	-	+	*NA*
Halling, 1996 (n = 28)	p53	Positive	67.9%	OS	+	*NA*								
Holtkamp, 2007 (n = 36)	MMP-13	Positive	58.0%	DFS	-									
Ikuta, 2014 (n = 30)	HA	>20%	30.0%	OS	+	n.s.	-	-	+	-	*NA*	-	*NA*	-
				DFS	+	HR 5.7 (1.2-26.4)	-	-	+	-	*NA*	-	*NA*	-
	HAS1	>20%	50.0%	OS	-									
				DFS	-									
	HAS2	>20%	59.1%	OS	-									
				DFS	-									
	HAS3	>20%	31.8%	OS	-									
				DFS	-									
Jia, 2019 (n = 30)	Decorin	Positive	46.7%	OS	+	*NA*								
Keizman, 2009	EGFR	High[c]	43.4%	OS	+	*NA*								
(n = 46)				Met	+	*NA*								
Kobayashi, 2006	CHFR	Low[d]	65.6%	OS	+	HR 4.8 (1.9-12.2)	-	-	-	-	+	-	*NA*	-
(n = 58)	Ki67	≥30%	26.0%	OS	+	n.s.	-	-	-	-	+	-	*NA*	-

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
Kolberg, 2015 (n = 62)	Survivin	≥1%	44.1%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	TK1	>10% or ≥1% + high intense	26.3%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	TOP2A	>10%	29.8%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	Combination	High risk[e]	30.4%	DSS	+	HR 4.6 (1.5-14.4)	-	-	-	-	*NA*	*NA*	-	-
Kourea, 1999 (n = 35)	p53	≥20%	28.6%	OS	-									
				Rec	-									
	pRB	≥3%	88.6%	OS	-									
				Rec	+	*NA*								
	p21	≥20%	45.7%	OS	-									
				Rec	-									
	p27	≥20%	54.3%	OS	+	*NA*								
				Rec	-									
	Cyclin D1	≥10%	28.6%	OS	-									
				Rec	-									
	Cyclin E	≥10%	42.4%	OS	-									
				Rec	-									
	Ki67	≥20%	58.8%	OS	-									
				Rec	-									

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
Krawczyk, 2019 (n = 26)	Survivin	High	69.2%	OS	-									
				DFS	-									
	Cyclin D1	High	50.0%	OS	+	HR 15.9 (2.0-125.0)	-	-	*NA*	-	+	-	-	*NA*
				DFS	+	HR 11.1 (2.8-47.6)	-	-	*NA*	-	-	-	-	*NA*
	Osteopontin	High	50.0%	OS	-									
				DFS	-									
	Fibronectin	High	61.5%	OS	-									
				DFS	-									
	p53	High	61.5%	OS	-									
				DFS	+	HR 3.2 (1.0-10.4)	-	-	*NA*	-	+	-	-	*NA*
LaFemina, 2013 (n = 105)	S100	Absent	*NA*	DSS	-									
Le Guellec, 2016 (n = 106)	S100	Absent	15.4%	OS	-									
				Rec	-									
				Met	-									
				DFS	-									
	MDM2	Positive	37.8%	OS	-									
				Rec	-									
				Met	-									
				DFS	-									
	Desmin	Positive	29.7%	OS	-									
				Rec	-									
				Met	-									
				DFS	-									

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
Leroy, 2001 (n = 12)	p53	Positive	50.0%	OS	-									
Lu, 2018 (n = 48)	ATRX	≤80%	65.1%	OS	+	HR 5.3 (1.4-20.4)[f]	-	*NA*	-	-	+	*NA*	+	*NA*
Meis, 1992 (n = 57)	S100	Absence	47.4%	OS	-									
Otsuka, 2018 (n = 97)	H3K27me3	<75%	65.5%	OS	+	n.s.	*NA*	-	-	*NA*	*NA*	*NA*	*NA*	-
Panse, 2017 (n = 29)	p-STAT3	>10%	51.7%	DSS	+	*NA*								
				DFS	-									
Pekmezci, 2017 (n = 39)	H3K27me3	<5%	43.6%	OS	-									
				DFS	-									
Skotheim, 2003 (n = 44)	TOP2A	>5%	83.3%	OS	+	*NA*								
				Met	+	*NA*								
	Ki67	>5%	57.5%	OS	-									
				Met	-									
Tabone, 2008 (n = 42)	EGFR	Positive	85.7%	OS	-									
Torres, 2011 (n = 55)	MET	% or intensity	*NA*	DSS	-									
	HGF	% or intensity	*NA*	DSS	-									
	p-MET	% staining	*NA*	DSS	+	HR 1.0 (1.0-1.1)	*NA*	*NA*	-	*NA*	*NA*	*NA*	*NA*	*NA*
	p53	Intensity	*NA*	DSS	+	HR 2.3 (1.2-4.5)	*NA*	*NA*	-	*NA*	*NA*	*NA*	*NA*	*NA*
	S100	Absent	*NA*	DSS	+	n.s.	*NA*	*NA*	-	*NA*	*NA*	*NA*	*NA*	*NA*

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
Vasconcelos, 2019 (n = 29)	MCD	High	33.3%	OS	-									
	MVD	High	33.3%	OS	+	HR 7.3 (1.4-38.5)	-	-	-	-	*NA*	*NA*	-	*NA*
	Ki67	High	33.3%	OS	-									
Wang, 2015 (n = 38)	S100	Absent	21.1%	OS	+	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
				Rec	+	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	Vimentin	Absent	14.7%	OS	-									
				Rec	-									
	GFAP	Absent	51.8%	OS	-									
				Rec	-									
	NSE	Absent	48.4%	OS	-									
				Rec	-									
	Ki67	≥20%	29.6%	OS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
				Rec	-									
	SMA	Absent	40.0%	OS	-									
				Rec	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	CD57	Absent	22.7%	OS	-									
				Rec	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
Wasa, 2008 (n = 22)	VEGF	≥25%	36.4%	OS	+	*NA*								
	MVD	*NA*	*NA*	OS	-									
Watanabe, 2001 (n = 49)	p53	>5%	28.6%	OS	-									
	Ki67	>25%	23.3%	OS	+	n.s.	-	-	-	-	-	-	*NA*	-
	MDM2	>5%	67.3%	OS	-									
	p21	>5%	71.4%	OS	-									

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
Yu, 2011 (n = 87)	SOX5	High[g]	38.3%	OS	-									
	FOXM1	High	41.5%	OS	+	HR 1.9 (1.1-3.3)	-	-	-	*NA*	+	*NA*	*NA*	*NA*
	Myc	High	32.5%	OS	+	n.s.	-	-	-	*NA*	+	*NA*	*NA*	*NA*
	p53	High	33.3%	OS	-									
Yuan, 2017 (n = 112)	S100	Absent	15.2%	OS	+	HR 6.6 (1.8-23.8)	-	-	-	-	+	-	-	*NA*
				DFS	+	HR 4.2 (1.5-12.3)	-	-	-	-	+	-	-	*NA*
	Ki67	≥20%	58.0%	OS	+	n.s.	-	-	-	-	+	-	-	*NA*
				DFS	+	HR 3.8 (1.7-8.5)	-	-	-	-	+	-	-	*NA*
	Vimentin	Absent	4.6%	OS	-									
				DFS	-									
	NF	Absent	52.2%	OS	-									
				DFS	-									
	GFAP	Absent	78.2%	OS	-									
				DFS	-									
Zhang, 2017 (n = 58)	CXCR4	High[h]	32.8%	OS	+	n.s.	-	*NA*	-	-	+	*NA*	-	*NA*
				DFS	-									
	CXCL12	High	55.2%	OS	-									
				DFS	-									
	Cyclin D1	High	27.6%	OS	-									
				DFS	-									

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
Zhou, 2016 (n = 63)	FGFR1	Low[i]	69.8%	OS	+	HR 2.8 (1.2-6.7)	-	-	-	-	-	*NA*	-	*NA*
				DFS	-									
	FGFR2	Low	*NA*	OS	-									
				DFS	-									
	FGFR4	High	82.3%	OS	-									
				DFS	+	n.s.	-	+	-	-	+	*NA*	-	*NA*
Zou, 2009 (n = 69)	S100	Absent	40.7%	DSS	+	HR 4.5 (2.0-12.1)[j]	-	-	-	-	-	*NA*	-	*NA*
				Rec	-									
	Ki67	%	*NA*	DSS	-									
	p53	Intensity	*NA*	DSS	+	HR 1.8 (1.0-3.3)	-	-	-	-	-	*NA*	-	*NA*
	VEGF	Intensity	*NA*	DSS	-									
	EGFR	Intensity	*NA*	DSS	+	*NA*								
	p-MEK	Intensity	*NA*	DSS	-									
				Genetic alterations										
Alaggio, 2013 (n = 35)	*BIRC5* mRNA	75 fold change	50.0%	OS	+	*NA*								
Brekke, 2010 (n = 46)	Xq	Loss	26.1%	DSS	+	HR 3.6 (1.6-8.3)	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	Xp	Loss	23.9%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	1q	Gain	34.8%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	2q	Gain	39.1%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	4q	Loss	17.4%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	5p	Gain	30.4%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	6q	Gain	30.4%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
	7q	Gain	37.0%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	10q	Loss	41.3%	DSS	+	HR 3.2 (1.4-7.7)	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	10p	Loss	41.3%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	16p	Gain	21.7%	DSS	+	HR 2.5 (1.0-6.2)	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	16q	Loss	34.8%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	20q	Gain	23.9%	DSS	+	n.s.	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	Combination	High risk[k]	63.0%	DSS	+	HR 11.0 (3.5-35.0)	*NA*	-	-	-	+	*NA*	*NA*	-
Danielsen, 2015 (n = 32)	*RASSF1A*	Methylated	62.5%	DSS	+	HR: 5.2 (1.4-19.4)[l]	*NA*	*NA*	-	-	-	*NA*	*NA*	*NA*
Høland, 2018 (n = 84)	*TP53*	Mutation	9.5%	DSS	+	*NA*								
		Gain	31.3%	DSS	-									
		Loss	9.5%	DSS	+	*NA*								
		LOH	44.6%	DSS	+	*NA*								
	MDM2	Amplification	6.0%	DSS	+	*NA*								
	Combination	High risk[m]	16.0%	DSS	+	HR 3.4 (1.4-8.3)	-	-	-	+	*NA*	*NA*	*NA*	*NA*
	Combination	≥-0.12 score[n]	66.7%	DSS	+	HR 4.0 (1.3-12.1)	-	-	-	-	*NA*	*NA*	*NA*	*NA*
Holtkamp, 2007 (n = 36)	p53Pro[72]	Polymorph	41.7%	Met	+	*NA*								
Holtkamp, 2008 (n = 37)	*CDKN2A*	Loss	56.8%	OS	-									
Kresse, 2008 (n = 7)	17q23.2-25.3	Gain	71.4%	OS	+	*NA*								
	TOP2A	High	*NA*	OS	-									
	ETV4	High	*NA*	OS	-									
	HOXB7	High	*NA*	OS	-									

Author, year (N patients)	Marker	Cut-off	Inc	Outcome	Uni	Multivariable (95% CI)	Other evaluated independent predictors							
							A	NF	Sz	St	M	D	R	G
	BIRC5	High	*NA*	OS	-									
	miR-142-3p	High	*NA*	OS	-									
	miR-142-5p	High	*NA*	OS	-									
	miR-301	High	*NA*	OS	-									
	miR-21	High	*NA*	OS	-									
	miR-338	High	*NA*	OS	-									
Nobeyama, 2016 (n = 20)	*MAGEA3*	Methylated	70.0%	OS	+	*NA*								
Yu, 2011 (n = 38)	*SOX5*	Gain	30.6%	OS	+	n.s.	-	-	+	*NA*	-	*NA*	*NA*	*NA*
	NOL1	Gain	25.0%	OS	+	n.s.	-	-	+	*NA*	-	*NA*	*NA*	*NA*
	FOXM1	Gain	30.6%	OS	+	n.s.	-	-	+	*NA*	-	*NA*	*NA*	*NA*
	CDK4	Gain	25.0%	OS	+	HR 4.2 (1.4-12.4)	-	-	+	*NA*	-	*NA*	*NA*	*NA*
		Amplification	15.2%	OS	+	HR 2.0 (1.0-4.0)	-	-	-	*NA*	+	*NA*	*NA*	*NA*
	ERBB2	Gain	*NA*	OS	-									
		Amplification	*NA*	OS	-									
	MYC	Gain	44.4%	OS	-									
		Amplification	*NA*	OS	-									
	TP53	Gain	*NA*	OS	-									
Zhou, 2016 (n = 52)	*FGFR1*	Low		OS	+	n.s.	-	-	-	-	-	*NA*	-	*NA*
				DFS	+	n.s.	-	+	-	-	+	*NA*	-	*NA*

a) In sporadic patient subpopulation of prognostic value

b) Preservation of p15 expression in combination with other inactivated genes was of prognostic value

c) Composite score is multiplication of intensity (score 0-3) and percentage of positive cells, >10 points was a high score

d) Composite score was scored by adding percentage of positive cells (score 0 = <10%, 1 = 10-49%, 2 = ≥50%) and intensity (score 0-3), ≤3 points was a low score

e) Combination risk profile low survivin + low TOP2A or high survivin + low TK1 = low risk, low survivin + high TOP2A or high survivin + high TK1 = high risk

f) Significance for NF1 patients only cohort, but not in sporadic patient cohort or overall cohort (n = 74)

g) Moderate intensity with >20% staining or >5% staining and strong intensity as highly positive

h) Composite score was scored by adding percentage of positive cells (score 0 = ≤10%, 1 = 11-25%, 2 = 26-50%, 3 = 51-75%, 4 = >75%) and intensity (score 0-3), ≥4 points was classified as high

i) Composite sore of adding staining (score 0 = <5% 0, 1 = 5-25%, 2 = 26-50%, 3 = 51-75%, 4 = >75%) and intensity (no = 0, yellow = 1, tan = 2, brown = 3), score >3 is high

j) S100 lost significance when p53 intensity was included in multivariate model

k) Loss from either Xq or 10q or gain at 16p (63.0%) after correction in multivariate model

l) Significance for NF1 patients only cohort, but not in sporadic patient cohort or overall cohort (n = 60)

m) Combination of either MDM2 amplification or TP53 abberation as high-risk group

n) Gene expression profile score

4E-BP1: eukaryotic translation initiation factor 4E-binding protein 1, A: age, CDK: cyclin dependant kinase, CHFR: checkpoint with forkhead-associated domain and ring finger, CI: confidence interval, COX-2: cyclooxygenase-2, CXCR4: C-X-C motif chemokine receptor 4, CXCL12: C-X-C motif chemokine ligand 12, D: tumour depth, DFS: disease-free survival, DSS: disease-specific survival, EGFR: epidermal growth factor receptor, ERK: extracellular signal-regulated kianses, FGFR: fibroblast growth factor receptor, FOXM1: forkhead box protein M1, G: tumour grade, GFAP: glial fibrillary acidic protein, H3K27me3: trimethylation of lysine 27 of histone H3, HA: hyaluronan, HAS: hyaluronan synthase, HIF: hypoxia-inducible factor, HPF: high-power field, HR: hazard ratio, Inc: percentage of population with 'positive' marker, M: metastasatic disease at diagnosis, MDM2: mouse double minute 2 homolog, MEK: mitogen-activated protein kinase kinase, Met: metastasis, MMP: matrix metalloproteinase, mTOR: mammalian target of rapamycin, MVD: microvessel density, N: number of patients analysed, NA: not available/analyzed, NF: neurofibromatosis type 1, n.s.: not significant, OR: odds ratio, OS: overall survival, p-: phosphorylated, R: surgical margin, RASSF1A: Ras association domain family member 1, isoform A, RB: retinoblastoma protein, Rec: recurrence, S6RP: ribosomal protein S6, SMA: smooth muscle actin, St: tumour site, STAT3: Signal transducer and activator of transcription 3, Sz: tumour size, TIMP-2: tissue inhibitor of metalloproteinase 2, TK1: thymidine kinase 1, TOP2A: topoisomerase 2-alpha, Uni: univariate analysis, VEGF: vascular endothelial growth factor

PART II

Development of Prediction Tools in STS: Identifying Patients at Risk and Predicting Oncological Outcome

Chapter 6

Non-Invasive Detection of Soft Tissue Sarcoma using Volatile Organic Compounds in Exhaled Breath: A Pilot Study

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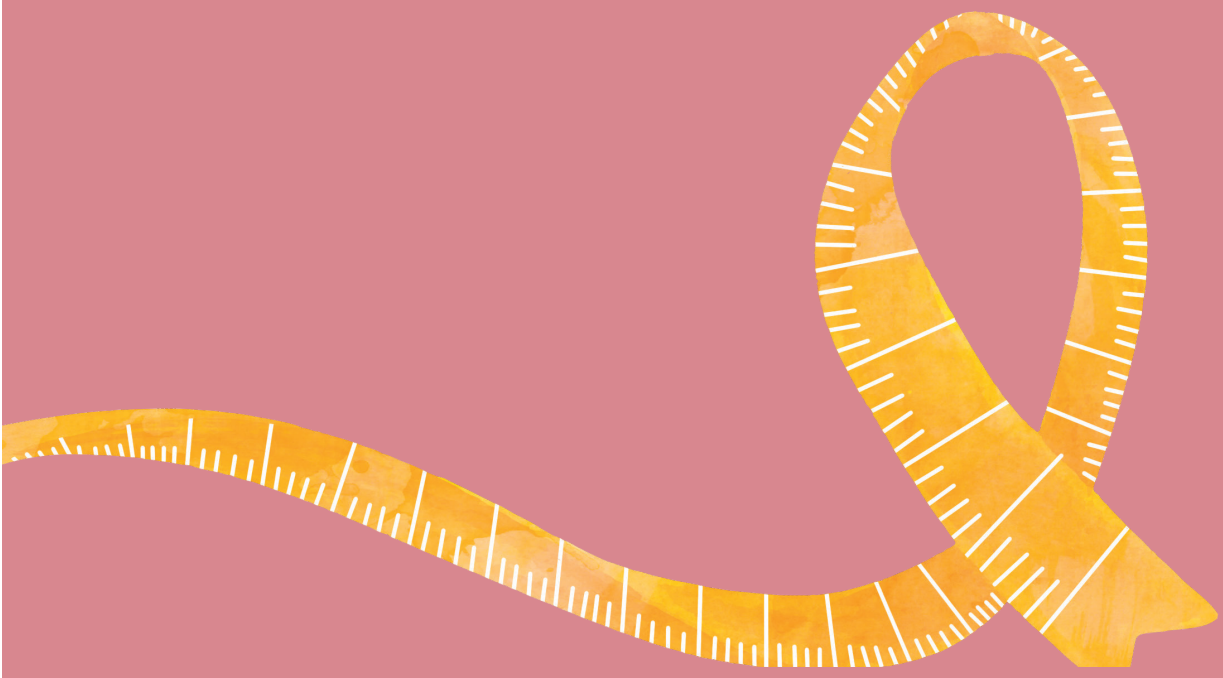

Abstract

Background

Volatile Organic Compounds (VOC) are widely investigated as a new diagnostic biomarker in medicine. The aim of this pilot study is to assess whether an electronic nose can detect patients with soft tissue sarcoma (STS) based on Volatile Organic Compound profiles in exhaled breath.

Methods

In this cross-sectional pilot study, patients with primary histologically proven STS were included for breath analysis from March 2018-2022. Persons matched on sex and age were included for the control group. Machine-learning techniques were used to develop the best fitting model. Ten-fold cross-validation was used for internal validation.

Results

Fifty-nine breath samples were collected (29 STS and 30 control). The final model yields an area under the curve of 0.85 with a sensitivity and specificity of 83% (95% CI 64-93) and 60% (95% CI 41-77), respectively.

Conclusion

This study suggests that exhaled VOC analysis could serve as a non-invasive diagnostic biomarker for the detection of STS with a good performance.

Introduction

Differentiating soft tissue sarcomas (STS) from benign soft tissue tumours is challenging in daily practice. The incidence of STS is less than 4.7 per 100,000 persons per year in Northern Europe (1) and it has been estimated that benign soft tissue tumours occur 300 times more often than their malignant counterparts. (2-4) Besides the rarity, STS often present as asymptomatic or unspecific lumps. These difficulties explain why STS are often thought to be benign. This results in frequently performed unplanned excisions, in which the STS is inadvertently and inadequately removed without an appropriate diagnosis, preoperative imaging or planning. (5) Referrals after unplanned excisions account for 8-53% of the new patients treated in sarcoma centres. (3, 6-11) These patients often require re-excision due to incomplete surgical margins. (12, 13)

Although core needle biopsy is an invasive procedure that is prone to various complications, it is the gold standard for differentiating STS from benign soft tissue tumours. Because benign tumours are very common, there is a serious need for novel non-invasive diagnostic tools that accurately detect patients with STS. Achievement of a higher pre-test probability for STS could reduce the number of unplanned excisions and re-excisions, but could also reduce the number of imaging assessments and biopsies during routine follow-up.

In the past years, Volatile Organic Compounds (VOC) were widely investigated as a new diagnostic biomarker in medicine. VOC profiles could be detected in breath, blood, saliva, semen, milk, faeces, urine and on the skin. (14) Several studies have been performed with a non-invasive electronic nose (eNose) in which VOC profiles were detected from exhaled breath. VOC analyses seem promising for the detection of several cancer types such as lung, breast, prostate, colorectal, head and neck carcinoma. (15-17) However, no studies have been performed investigating the discriminative ability of the eNose for STS. Therefore, the aim of this pilot study is to assess whether the eNose can discriminate between patients with and without STS based on VOC profiles in exhaled breath.

Methods

Study design

This prospective proof-of-principle study was conducted in a specialized sarcoma centre outpatient clinic in a tertiary hospital in the Netherlands (Leiden University Medical Centre) between March 2018 and March 2022. Ethical approval was obtained by the institutional review board prior to the study (P18.046). All study participants provided

written informed consent before breath testing. The measurements were performed in parallel with the regular diagnostic work-up. No formal sample size calculation was performed for this pilot study. Based on previous studies with an electronic nose, a sample size of 25 participants per study arm was considered sufficiently powered for a pilot study. (18, 19) The primary outcome of this pilot study was the discriminative ability (Area Under the ROC Curve) of the VOC profiles recorded by the eNose.

Participants

Patients who were referred to our outpatient clinic for suspected primary STS were approached to participate in this study. Patients were included in this study if they had a histologically proven primary high-grade STS. Patients were excluded if they were younger than 18 years, had a history of cancer or chronic respiratory conditions (e.g., COPD or asthma), were previously treated with radiotherapy and/or chemotherapy, received any prior treatment for STS, or were diagnosed with distant metastasis within 3 months after inclusion. Also, patients who did not complete the breath test were excluded. Individuals with no suspicion for STS who visited our outpatient clinic for other conditions, or accompanied a patient to our outpatient clinic, and employees at our department were asked to participate in this study as healthy controls. Individuals with a suspected STS who turned to be a benign tumour (e.g., schwannoma, lipoma, haemangioma) were excluded in this analysis. The control group was matched to our STS population based on age and sex in a 1:1 ratio. The same exclusion criteria were applied to the control group. In addition, we performed a secondary analysis with less stringent inclusion criteria in order to expand the sample size. In this analysis we also included patients with a low-grade STS in the sick group and patients with a rejected STS in the control group.

Materials and study procedure

The eNose used in this study (Aeonose, The eNose Company, Zutphen, The Netherlands) is a handheld, battery-powered electronic nose which enables to analyse VOCs. Participants were instructed to breathe through a disposable connecting mouthpiece for 5 minutes. The mouthpiece contained a carbon filter, and a nose clip was placed on the nose of the participants to avoid entry of non-filtered air during the measurement to eliminate exogenous influences on VOC. In addition, the mouthpiece contained a high-efficiency particulate air (HEPA) filter and one-way valves to prevent viral and bacterial contamination of the device. In the first 2 minutes of each measurement the lungs were rinsed with clean filtered air to further eliminate exogenous VOCs. During the remaining 3 minutes the exhaled breath was guided over three micro hotplate metal-oxide sensors with different material properties. The hotplate was periodically heated between 260- and 340-degrees Celsius simulating multiple identical sensors that are operating at different temperatures. The VOCs in

the exhaled breath induce a redox reaction on the metal-oxide sensor surfaces, causing a conductivity change. These changes in conductivity over time result in a unique VOC profile for each participant. The total measurement took 15 minutes, consisting of 5 minutes breathing followed by 10 minutes of regeneration of the eNose. **Figure 1 of the appendix** depicts the eNose and test setup.

For the measurement all participants were asked to abstain from food, drink (except water), and smoking for at least 3 hours prior to the study visit to minimize exogenous VOCs. (20) Tumour characteristics (histological subtype and tumour grade) and medical history (previous malignancies and chronic respiratory conditions) were collected from clinical records.

Statistical analysis

Baseline characteristics were described with proportions for categorical variables and means with standard deviations or medians with interquartile ranges (IQRs) for continuous variables. Differences in continuous variables were assessed with the Mann-Whitney U test. Differences in categorical variables were assessed with the Pearson's Chi-square test.

An eNose measurement resulted in a time series of conductivity values for each sensor. Multiple machine learning models were built using different sensor combinations and classifiers. Data compression was performed using a Tucker3-like tensor decomposition technique. While applying 10-fold cross validation, models were ranked on AUC (Area Under the Curve). The validation results were averaged over the ten rounds, resulting in a combined AUC. The Random Forest classifier turned out to be most favourable. Data compression and data analyses were integrated in a proprietary software program (Aethena, The eNose Company, Zutphen, the Netherlands). Descriptive statistics were performed in R (version 4.1.2). (21)

A p-value ≤0.05 was considered statistically significant. Results from the final models were described by means of the most optimal AUC with corresponding sensitivity and specificity with 95% confidence intervals (CI). For each analysis, two cut-off values for the predicted response value of the final model were presented with corresponding sensitivity and specificity. The cut-off value was set manually at a predictive value of 0.0 and at a value at which the sensitivity was maximized with an acceptable specificity (≥50%). The predicted response value for the STS and control population was presented in a scatterplot.

Results

Twenty-five patients with high-grade STS and 25 controls, matched on age and sex, were included for the first analysis. For the second analysis with less stringent inclusion criteria, 29 patients with STS and 30 controls were included. Baseline demographics and clinical characteristics are presented in **Table 1**.

Figure 1A depicts the receiver operating characteristic (ROC) curve of model 1 with a fair discriminative ability with an AUC of 0.78. **Figure 1B** depicts a scatterplot of the predicted value of each measurement of model 1. Setting the predictive value at 0.0 resulted in a sensitivity and specificity of 72% (95% CI 50-87) and 76% (95% CI 54-90), respectively. A threshold of -0.3 resulted in a sensitivity and specificity of 84% (95% CI 63-95) and 52% (95% CI 32-73), respectively. **Figure 2** depicts the ROC curve (A) and scatterplot (B) of model 2 with less stringent inclusion criteria. This model showed that an increased sample size resulted in a better discriminative ability with an AUC of 0.85. At a threshold of 0.0, the sensitivity and specificity were 72% (95% CI 53-87) and 90% (95% CI 72-97), respectively. A threshold of -0.2 resulted in a sensitivity and specificity of 83% (95% CI 64-93) and 60% (95% CI 41-77), respectively. In the **appendix** a scatterplot of individual predicted values stratified by histologic subtype is presented (**Figure 2**).

Table 1. Baseline characteristics

	Model 1			Model 2		
	STS (N=25)	**Control (N=25)**	***P***	**STS (N=29)**	**Control (N=30)**	***P***
Age (in years)			0.669			0.679
Median [IQR]	57 [39-65]	54 [44-61]		56 [39-63]	59 [45-62]	
Sex			1			0.693
Female	8 (32.0%)	8 (32.0%)		11 (37.9%)	10 (33.3%)	
Male	17 (68.0%)	17 (68.0%)		18 (62.1%)	20 (66.7%)	
Histological subtype						
LPS	6 (24.0%)	-		6 (20.7%)	-	
LMS	2 (8.0%)	-		3 (10.3%)	-	
MFS	5 (20.0%)	-		7 (24.1%)	-	
MPNST	5 (20.0%)	-		5 (17.2%)	-	
SS	2 (8.0%)	-		2 (6.9%)	-	
UPS	2 (8.0%)	-		2 (6.9%)	-	
Other	3 (12.0%)	-		4 (13.8%)	-	
Tumour size (in mm)						
Median [IQR]	60 [46-84]	-		60 [46-84]]	-	
Tumour grade						
1	0 (0.0%)	-		4 (13.8%)	-	
2	14 (56.0%)	-		14 (48.3%)	-	
3	8 (32.0%)	-		8 (27.6%)	-	
High-grade not otherwise specified	3 (12.0%)	-		3 (10.3%)	-	
Location						
Extremity	21 (84.0%)	-		24 (82.8%)	-	
Trunk wall	3 (12.0%)	-		4 (13.8%)	-	
Uterus	1 (4.00%)	-		1 (3.45%)	-	

IQR: interquartile range, LPS: liposarcoma, LMS: leiomyosarcoma, MFS: myxofibrosarcoma, MPNST: malignant peripheral nerve sheath tumour, SS: synovial sarcoma, UPS: undifferentiated pleomorphic sarcoma

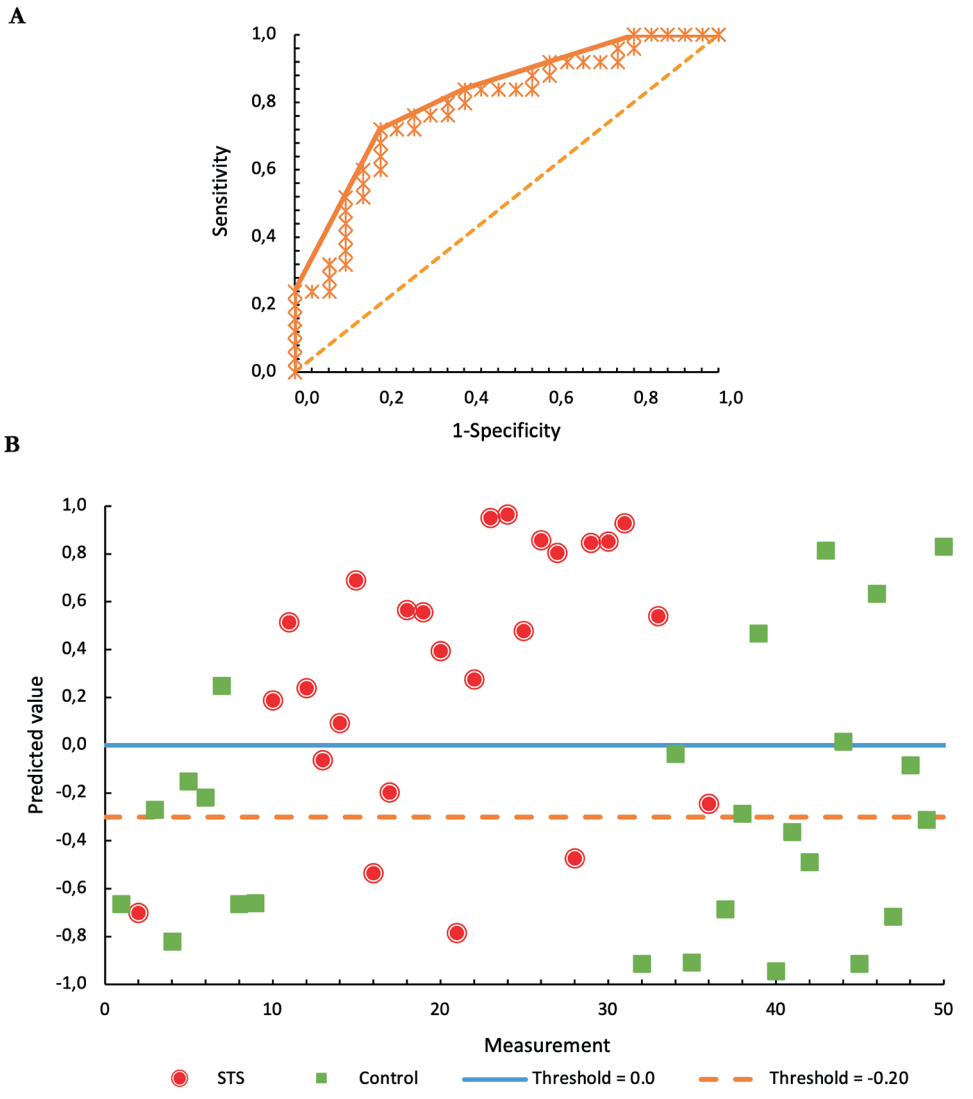

Figure 1A. Receiver operating characteristic curve for the best fit of model 1 (AUC: 0.78) **B.** Scatterplot of individual predicted values based on the cross-validated model 1.

The red circles represent patients with STS. The green squares represent the controls. AUC: Area under the curve, STS: Soft tissue sarcoma

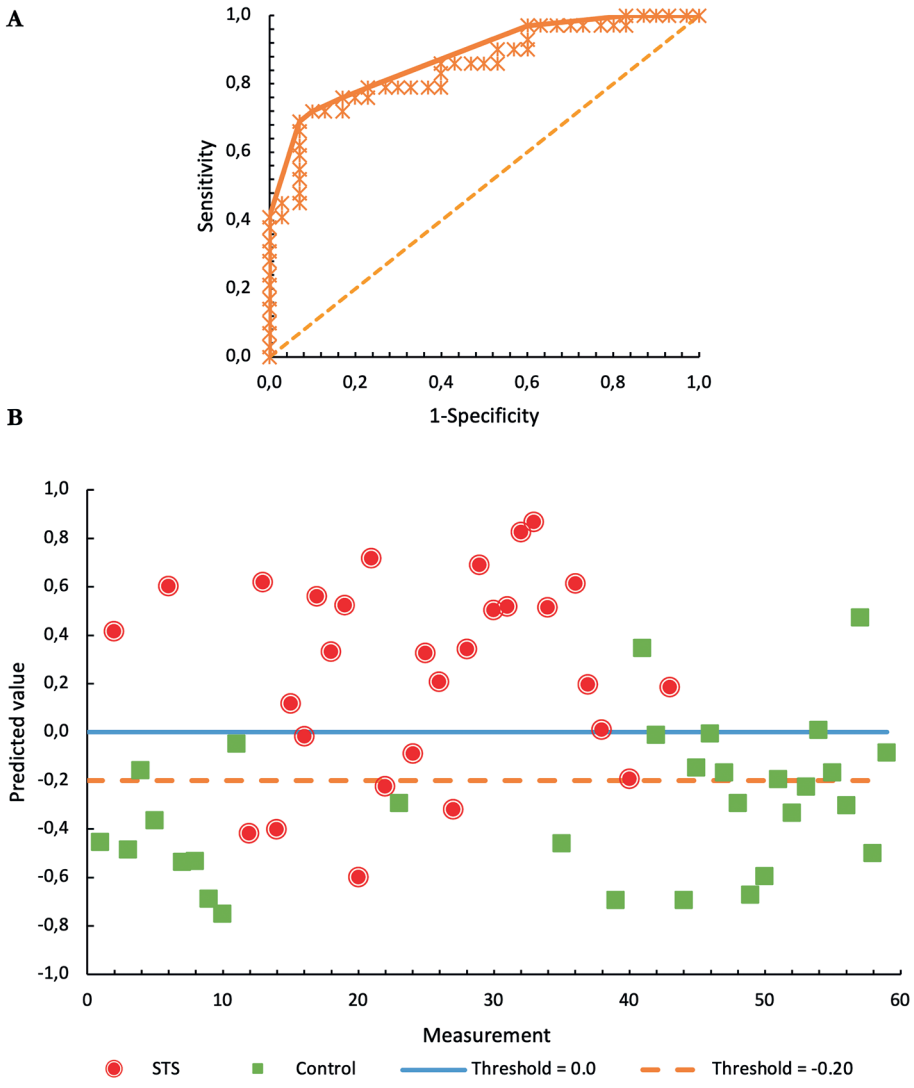

Figure 2A. Receiver operating characteristic curve for the best fit of model 2 (AUC: 0.85) **B.** Scatterplot of individual predicted values based on the cross-validated model 2.

The red circles represent patients with STS. The green squares represent the controls. AUC: Area under the curve, STS: Soft tissue sarcoma

Discussion

In this proof-of-principal study, we examined that the eNose could well distinguish patients with and without STS, suggesting that exhaled VOC analysis with eNose could become a promising non-invasive diagnostic tool to achieve a higher pre-test probability for STS, and potentially reduce the number of unplanned excisions, re-excisions, and biopsies. With an AUC of 0.85 of the second model and a corresponding sensitivity and specificity of 83% and 60%, respectively, the discriminative ability could be considered good. Larger multicentre studies are needed to confirm current findings, improve accuracy, and extend the validity of the current models.

In the last years, several phase I studies have demonstrated the diagnostic ability of VOC patterns in exhaled breath for several cancer types. (15-17) No studies so far have assessed the diagnostic performance of VOC profiles as diagnostic biomarker for STS. VOCs are a group of organic carbon and hydrogen-containing compounds that are found in various cellular functions such as oxidative stress and energy metabolism. Oxidative stress and altered cellular energy metabolism have been implicated in the pathophysiology of cancer in order to support continuous cell growth and proliferation. (22, 23) Changes in VOC concentrations reflect these altered metabolic and pathophysiological processes in the human body. (24) In breath there are almost 1,500 VOCs reported. (14) For most of the VOCs, the biochemical process for their production remains unknown. Several studies have shown that different cancer types and diseases reveal different VOC profiles, suggesting that VOC profiles could be diagnostic biomarkers for a broad range of diseases. (25, 26) Analysis of VOCs in exhaled breath is not yet implemented in clinical practice for any of the studied diseases. (15, 16)

Some limitations of this pilot study must be overcome in future studies. This study showed an overall good discriminative ability of the eNose. However, due to the limited sample size, the machine learning models built on our data could partially be based on artefacts in the data (e.g., due to contamination of exogenous VOCs) instead of true differences in VOC profile that were caused by the pathophysiology of the malignancy. Therefore, future larger studies are needed to update the models and externally validate the models. As shown in our second model, the discriminative ability of the model might even further improve with larger sample sizes. In most diagnostic studies, such as this study, the primary target is endogenous VOCs. However, human breath contains a mixture of endogenous and exogenous VOCs. Exogenous VOCs could arise from room air volatile, but also dietary habits and medication could influence the exhaled VOC profiles. (24, 27) In a large cohort of healthy volunteers, smoking behaviour, and to a lesser extent age, BMI, and gender influence VOC profiles in the

general population. (28) To minimize the effect of these influencing factors, we have matched the STS population with the control group by age and gender, performed all eNose measurements in the same testing area, and asked participants to abstain from food, drink, and smoking for 3 hours before testing. Furthermore, as radiotherapy and chemotherapy cause oxidative stress, inflammation and tissue damage, participants who received these treatments in history were excluded. (29, 30) We did not match patients based on BMI and other influencing factors, such as smoking status and comorbidities, because of the small sample size. The likelihood that these and other (unknown) influencing factors were not well distributed between the STS and control group is higher than in larger cohorts. For future studies, standardisation of the study procedure and breath collection, especially for training models, is crucial. Furthermore, studies should include controls from the same target population as the STS population (e.g., benign soft tissue tumours) to inform clinical application and should be externally validated in other target populations to assess generalizability of the models. In this pilot study only internal cross-validation was performed.

This proof-of-concept study aimed to assess the feasibility of an eNose for detection of STS at the beginning of a patient's work-up, when referred to a sarcoma centre with a primary nonspecific tumour of the soft tissue. The reported results were based on maximizing the sensitivity with acceptable specificity. Depending on the use of the eNose in clinical practice other cut-off values might be preferable. In a primary or secondary health care setting, the prevalence of benign soft tissue tumours is much higher than the prevalence of STS. The physician needs to decide whether to treat the tumour as a benign tumour or to refer the patient to a tertiary sarcoma centre for biopsy, which is the gold standard for diagnosis STS. (12, 31) This decision is nowadays based on physical examination and imaging. However, the large number of unplanned excisions in patients with STS reflects the inaccuracy of the current diagnostic work-up. (3, 6-11) Patients with asymptomatic benign tumours do often not need any treatment, while patients with primary STS need appropriate treatment in a sarcoma centre with an oncological resection with wide surgical margins and often (neo)adjuvant therapy. (12, 31) As a core needle biopsy is an invasive procedure and benign tumours are very common in this setting, not all patients with a nonspecific tumour of the soft tissue get a biopsy. A non-invasive diagnostic tool, such as a breath test, could help to decide which patient should get a biopsy in a tertiary sarcoma centre. In this case, maximizing the sensitivity, in order to minimize the risk of untreated or unplanned excisions for STS (false negatives), at the expense of more false positives referred to a sarcoma centre, would be clinically most desirable. Especially, in superficial and small STS the share of unplanned excisions is high. (6, 8-11) Therefore, the use of a non-invasive breath test in this target population and setting seems most promising and desirable.

Besides the use of an eNose as pre-test at the beginning of each patient's work-up to decide whether further diagnostic tests, such as a biopsy are needed, the eNose could also play a role in monitoring the response to cancer therapy, surveilling patients after successful treatment or differentiating between high- and low-risk STS. It is likely that for each application different VOC models with different cut-off values need to be built and validated. Furthermore, further studies are needed to assess the minimum detectable tumour volume for the eNose.

Conclusion

This study suggests that VOC in exhaled breath could become a new diagnostic biomarker for the detection of STS. Future studies are needed to validate these promising preliminary findings before VOC analyses could be incorporated in clinical practice.

References

1. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. European journal of cancer (Oxford, England : 1990). 2013;49(3):684-95.
2. Kang S, Han I, Lee SA, Cho HS, Kim HS. Unplanned excision of soft tissue sarcoma: the impact of the referring hospital. Surgical oncology. 2013;22(2):e17-22.
3. Fiore M, Casali PG, Miceli R, Mariani L, Bertulli R, Lozza L, et al. Prognostic effect of re-excision in adult soft tissue sarcoma of the extremity. Annals of surgical oncology. 2006;13(1):110-7.
4. Tedesco NS, Henshaw RM. Unplanned Resection of Sarcoma. J Am Acad Orthop Surg. 2016;24(3):150-9.
5. Giuliano AE, Eilber FR. The rationale for planned reoperation after unplanned total excision of soft-tissue sarcomas. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1985;3(10):1344-8.
6. Smolle MA, Tunn PU, Goldenitsch E, Posch F, Szkandera J, Bergovec M, et al. The Prognostic Impact of Unplanned Excisions in a Cohort of 728 Soft Tissue Sarcoma Patients: A Multicentre Study. Annals of surgical oncology. 2017;24(6):1596-605.
7. Chandrasekar CR, Wafa H, Grimer RJ, Carter SR, Tillman RM, Abudu A. The effect of an unplanned excision of a soft-tissue sarcoma on prognosis. J Bone Joint Surg Br. 2008;90(2):203-8.
8. Nakamura T, Kawai A, Hagi T, Asanuma K, Sudo A. A comparison of clinical outcomes between additional excision after unplanned and planned excisions in patients with soft-tissue sarcoma of the limb : a propensity matching cohort study. The bone & joint journal. 2021;103-b(12):1809-14.
9. Zaidi MY, Ethun CG, Liu Y, Poultsides G, Howard JH, Mogal H, et al. The impact of unplanned excisions of truncal/extremity soft tissue sarcomas: A multi-institutional propensity score analysis from the US Sarcoma Collaborative. Journal of surgical oncology. 2019;120(3):332-9.
10. Charoenlap C, Imanishi J, Tanaka T, Slavin J, Ngan SY, Chander S, et al. Outcomes of unplanned sarcoma excision: impact of residual disease. Cancer Med. 2016;5(6):980-8.
11. Liang Y, Guo TH, Xu BS, Hong DC, Qiu HB, Zhou ZW, et al. The Impact of Unplanned Excision on the Outcomes of Patients With Soft Tissue Sarcoma of the Trunk and Extremity: A Propensity Score Matching Analysis. Front Oncol. 2020;10:617590.
12. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2021;32(11):1348-65.
13. Sacchetti F, Alsina AC, Morganti R, Innocenti M, Andreani L, Muratori F, et al. Re-excision after unplanned excision of soft tissue sarcoma: A systematic review and metanalysis. The rationale of systematic re-excision. J Orthop. 2021;25:244-51.
14. Drabińska N, Flynn C, Ratcliffe N, Belluomo I, Myridakis A, Gould O, et al. A literature survey of all volatiles from healthy human breath and bodily fluids: the human volatilome. J Breath Res. 2021;15(3).
15. Hanna GB, Boshier PR, Markar SR, Romano A. Accuracy and Methodologic Challenges of Volatile Organic Compound-Based Exhaled Breath Tests for Cancer Diagnosis: A Systematic Review and Meta-analysis. JAMA Oncol. 2019;5(1):e182815.
16. Oakley-Girvan I, Davis SW. Breath based volatile organic compounds in the detection of breast, lung, and colorectal cancers: A systematic review. Cancer Biomark. 2017;21(1):29-39.

17. Waltman CG, Marcelissen TAT, van Roermund JGH. Exhaled-breath Testing for Prostate Cancer Based on Volatile Organic Compound Profiling Using an Electronic Nose Device (Aeonose™): A Preliminary Report. Eur Urol Focus. 2020;6(6):1220-5.
18. Schuermans VNE, Li Z, Jongen A, Wu Z, Shi J, Ji J, et al. Pilot Study: Detection of Gastric Cancer From Exhaled Air Analyzed With an Electronic Nose in Chinese Patients. Surg Innov. 2018;25(5):429-34.
19. van de Goor R, Hardy JCA, van Hooren MRA, Kremer B, Kross KW. Detecting recurrent head and neck cancer using electronic nose technology: A feasibility study. Head Neck. 2019;41(9):2983-90.
20. Dragonieri S, Quaranta VN, Buonamico E, Battisti C, Ranieri T, Carratu P, et al. Short-Term Effect of Cigarette Smoke on Exhaled Volatile Organic Compounds Profile Analyzed by an Electronic Nose. Biosensors (Basel). 2022;12(7).
21. R Development Core Team. R: A language and environment for statistical computing. 3.6.3 ed. Vienna, Austria: R Foundation for Statistical Computing; 2010.
22. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative Stress in Cancer. Cancer Cell. 2020;38(2):167-97.
23. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022;12(1):31-46.
24. Issitt T, Wiggins L, Veysey M, Sweeney ST, Brackenbury WJ, Redeker K. Volatile compounds in human breath: critical review and meta-analysis. J Breath Res. 2022;16(2).
25. Peng G, Hakim M, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, et al. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. Br J Cancer. 2010;103(4):542-51.
26. van Hooren MR, Leunis N, Brandsma DS, Dingemans AC, Kremer B, Kross KW. Differentiating head and neck carcinoma from lung carcinoma with an electronic nose: a proof of concept study. Eur Arch Otorhinolaryngol. 2016;273(11):3897-903.
27. Salman D, Ibrahim W, Kanabar A, Joyce A, Zhao B, Singapuri A, et al. The variability of volatile organic compounds in the indoor air of clinical environments. J Breath Res. 2021;16(1).
28. Blanchet L, Smolinska A, Baranska A, Tigchelaar E, Swertz M, Zhernakova A, et al. Factors that influence the volatile organic compound content in human breath. J Breath Res. 2017;11(1):016013.
29. Salman D, Eddleston M, Darnley K, Nailon WH, McLaren DB, Hadjithelki A, et al. Breath markers for therapeutic radiation. J Breath Res. 2020;15(1):016004.
30. Phillips M, Byrnes R, Cataneo RN, Chaturvedi A, Kaplan PD, Libardoni M, et al. Detection of volatile biomarkers of therapeutic radiation in breath. J Breath Res. 2013;7(3):036002.
31. Von Mehren M, Kane JM, Agulnik M, Bui MM, Ganjoo KN, George S, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. 2018;16(5):536-63.

Appendix

Figure 1. The eNose and test setup

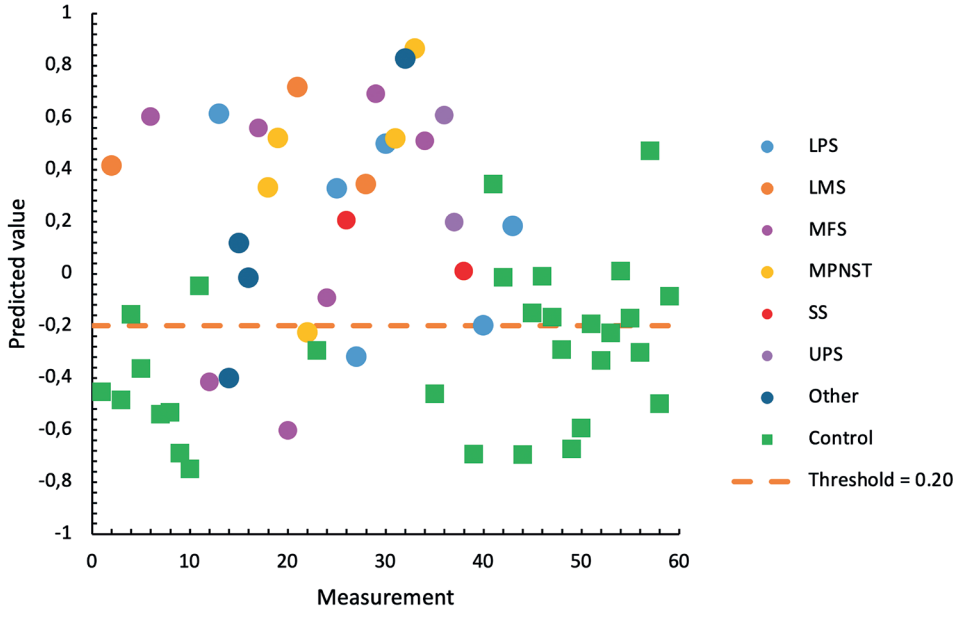

Figure 2. Scatterplot of individual predicted values based on the cross-validated model 2 stratified by histologic subtype.

The circles represent patients with STS. The green squares represent the controls. LPS: liposarcoma, LMS: leiomyosarcoma, MFS: myxofibrosarcoma, MPNST: malignant peripheral nerve sheath tumour, SS: synovial sarcoma, UPS: undifferentiated pleomorphic sarcoma

Chapter 7

Survival after Resection of Malignant Peripheral Nerve Sheath Tumours: Introducing and Validating a Novel Type-Specific Prognostic Model

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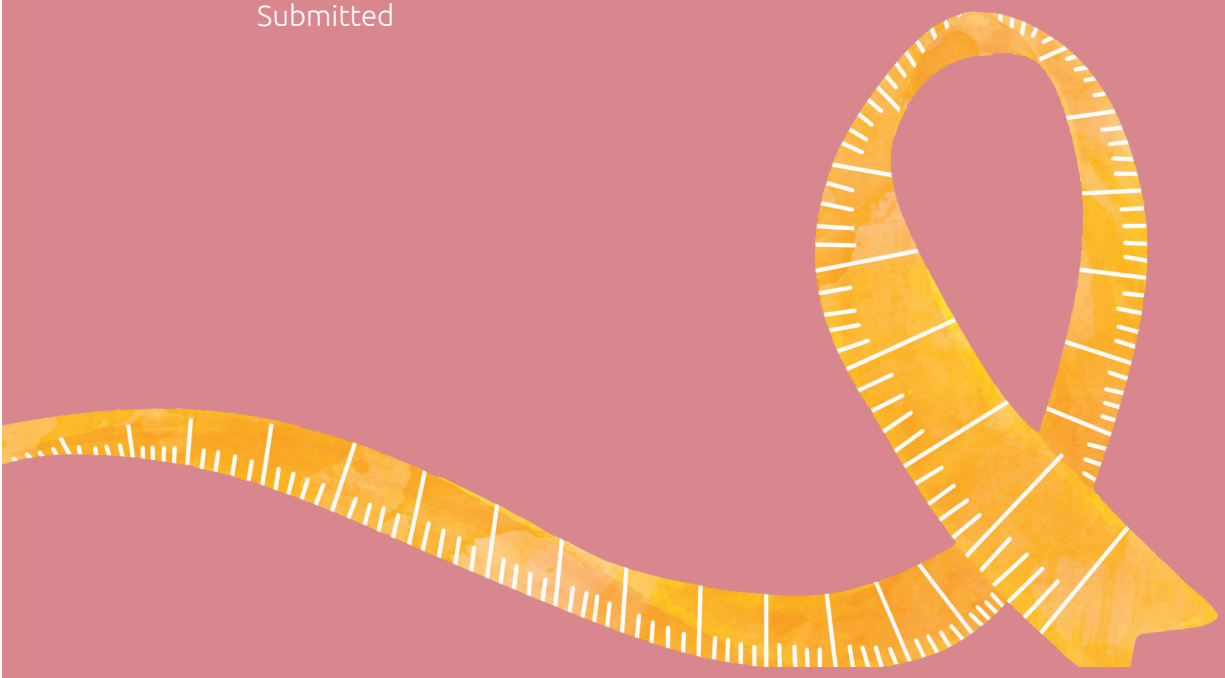
Submitted



Abstract

Background

This study aimed to assess the performance of currently available risk calculators in a cohort of patients with MPNST and to create an MPNST-specific prognostic model including type-specific predictors for overall survival (OS).

Methods

This is a retrospective multicentre cohort study of patients with MPNST from eleven sarcoma centres. All patients diagnosed with primary MPNST who underwent macroscopically complete surgical resection from 2000-2019 were included in this study. A multivariable Cox proportional hazard model for OS was estimated with pre-specified predictors (age, grade, size, NF-1 status, triton status, depth, tumour location and surgical margin). Model performance was assessed for the Sarculator and PERSARC calculators by examining discrimination (C-index) and calibration (calibration plots and observed-expected statistic; O/E-statistic). Internal-external cross-validation by different regions was performed to evaluate the generalizability of the model.

Results

A total of 507 patients with primary MPNSTs were included from 11 centres in 7 regions. During follow-up (median 8.7 years), 211 patients died. The C-index was 0.60 (95% CI 0.53-0.67) for both Sarculator and PERSARC. The MPNST-specific model had a pooled C-index of 0.69 (95%CI 0.65-0.73) at validation, with adequate discrimination and calibration across regions.

Conclusion

The MPNST-specific MONACO model can be used to predict 3-, 5-, and 10-year OS in patients with primary MPNST who underwent macroscopically complete surgical resection. Further validation may refine the model to inform patients and physicians on prognosis and support them in shared decision-making.

Introduction

Prognostic tools are important instruments for clinical decision making in soft tissue sarcomas (STS). STS is a heterogeneous group of malignant tumours with more than 100 different histological types that can affect patients of all age groups. (1) Given the heterogeneity of prognosis within the STS spectrum, several classification systems have been developed to classify patients into different risk groups. Traditionally, the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system and American Joint Committee of Cancer (AJCC) staging system were used to classify STS patients in different risk groups. (2, 3) However, in the last decade several new prognostic tools have been developed incorporating patient, tumour and treatment characteristics that generate individual prognosis for patients with STS. Two widely used prognostic tools for STS of the extremities are Sarculator and PERSARC. (4, 5) These tools can be used for the most common histological types such as leiomyosarcoma, liposarcoma, undifferentiated pleomorphic sarcoma, synovial sarcoma, and malignant peripheral nerve sheath tumours (MPNSTs). Although applicable to a wide range of STSs, these tools are limited to general predictors and do not incorporate type-specific prognostic factors.

MPNST is a rare and aggressive sarcoma type that accounts for 2-6% of all STS. (6-8) While most STS arise de novo, MPNSTs can be associated with neurofibromatosis type 1 (NF-1). (1) Approximately 30-50% of the MPNSTs are NF-1-associated. (9, 10) The NF1 gene is commonly affected in MPNSTs which causes loss-of-function of neurofibromin and inhibition of RAS oncogenes. (11) Several studies have shown that NF-1 status is a negative predictor for overall survival (OS) and distant metastasis (DM). (10, 12) In addition, MPNSTs can present with partial rhabdomyoblastic differentiation (triton tumour) which appear to have a poorer prognosis compared with conventional MPNSTs. (13)

Considering that, in contrast to other STS types, MPNSTs can occur in patients with NF-1 and can present with partial rhabdomyoblastic differentiation, one may argue that the commonly used generic prognostic tools for STS, such as Sarculator and PERSARC (4, 5), could be improved by MPNST-specific predictors such as NF-1 and triton status. As shown in a recent study, Sarculator is a good model to predict survival in patients from the United States with resected STS of the extremities. (14) However, the performance in patients with MPNSTs was poorer than in patients with other histological types. (14) Furthermore, the Sarculator models were only built on patients of 18 years and older with a retroperitoneal or extremity STS and PERSARC was only built on patients of 18 years and older with high-grade extremity STS. (4, 5, 15) While, around 50% of the MPNSTs is located outside the extremities and

retroperitoneum and approximately 10% of the patients is younger than 18 years old. (16)

The first aim of this study is to assess the performance of both Sarculator and PERSARC in an external cohort of MPNST patients. Furthermore, we extend and update these models by developing an MPNST-specific prognostic tool that is can be used for a wider range of patients with primary MPNST.

Methods

Study design

A retrospective multicentre international cohort study, the MPNST Oncological And Clinical Outcome Consortium (MONACO), was undertaken after approval of the institutional review boards of all included centres. Patients from eleven secondary or tertiary sarcoma centres diagnosed with histologically proven primary MPNST who were surgically treated with curative intent from 1 January 2000 to 31 December 2019 were included in this study. The following patients were excluded: patients with macroscopic residual disease (R2) after definitive surgery; patients with incorrectly registered time-to-event outcomes; patients with local recurrence (LR) who were previously resected elsewhere; patients with synchronous metastasis, defined as distant disease before date of definitive surgery. The list of participating centres is available in **Appendix A**.

Study procedure

Clinical and pathological data were retrieved from medical records or from existing prospective sarcoma databases. All included centres adhere to the clinical guidelines of the European Society for Medical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN) for STS. (17, 18) Follow-up usually consisted of clinical examination and imaging (CT scan or X-ray of the chest and MRI for local control) once every 4 months for 2 years, every 6 months up to 5 years after definitive treatment, and thereafter yearly.

External validation and extension approach

To validate and extend the prognostic models for MPNSTs, we undertook three steps. First, we validated the original PERSARC and Sarculator models for STS of the extremities (eSTS) in a subset of our cohort. This subset included all patients with primary high-grade (II/III) MPNST of the extremities. Model performance was assessed at 5 years from definitive surgery. Secondly, the original models were updated and extended by using the original predictors plus the MPNST-specific predictors. In this model, patients were included without eligibility restrictions on age, location, and

grade. Finally, the extended model was internally-externally cross-validated across 7 regions. This means that each region was left out once while models were developed in the remaining 6 regions. (19) As this split based on regions is not random, it qualifies as external validation. (19) We used regions instead of centres to ensure sufficient number of events within each split. A list of the specified regions is available in **Appendix A**.

Time-to-event was defined as the time interval between date of definitive surgery (T=0) and death from any cause. The outcomes of interest were OS at 3, 5, and 10 years.

The American Joint Committee on Cancer criteria were used for building a high-quality extended prognostic model. (20) The Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement was followed for reporting the validation and extension of the prediction models (**Appendix B** provides TRIPOD checklist). (21)

Candidate predictor variables

The prognostic factors included in the model were pre-specified based on Sarculator, PERSARC and literature review for MPNST-specific predictors. (10, 12, 13, 16, 22, 23) The included predictors were: age, grade, size, NF-1 status, triton status, depth, tumour location and surgical margin. All possible interaction terms with NF-1, location, and triton, were considered clinically plausible.

Age was determined as age at the time of diagnosis. Grade was based on the FNCLCC grading criteria (grade I, II, and III). (2) Tumour size was defined as the largest diameter (in cm) on imaging or based on pathology report if imaging was not available. A tumour was categorized as NF-1-associated by confirmed genetic testing of an NF1 mutation or by clinical evaluation. (24) Triton status was extracted from pathological reports and was concluded either when stated as such in the report or when MPNST with rhabdomyoblastic differentiation was reported. Depth was assessed on imaging and categorized as superficial or deep in relation to the investing fascia. Tumour location was categorized as extremity (including plexus), central (including thorax, abdomen, pelvis, retroperitoneal, or head and neck. Tumour margins were classified as negative (R0) or microscopically positive (R1) based on pathology reports. Macroscopically incomplete resections (R2) were excluded. The assessors of the predictors were inherently blinded for the outcome (death) due to the longitudinal nature of this study. OS was defined as the time interval between definitive surgery and date of death or date of last follow-up.

Model validation and extension

No formal sample size calculation was performed. All available data were used to maximise the robustness of our analyses. We ensured that we had at least 10 events per parameter for modelling pre-specified predictors in our full model. (25, 26) We determined the amount of optimism of the final model using bootstrap resampling (1000 replications). (25, 26) Shrinkage of regression coefficients was also estimated with this bootstrap validation procedure to improve predictions in future patients by preventing too extreme predictions due to overfitting. (25)

Multivariable Cox proportional hazard models were used for OS. The proportional hazard assumption was assessed visually with the Schoenfeld residuals. The possible non-linearity of the continuous variables age and size was modelled using restricted cubic splines (4 knots) in initial univariate analyses. Subsequently, we used simple parametric transformations, based on visual assessment. (25, 26) The chosen transformation was based on visual inspection and supported by Akaike information criterion (AIC), which penalizes for model complexity. The full model included all pre-specified predictors, the selected parametric transformation for the continuous variables and the potential interaction terms. All clinically plausible interactions were tested using a global test followed by individual testing if the global test was significant. (26) A p-value ≤0.20 was considered as threshold for the selection of interaction terms. (27)

To make efficient use of the available data, multiple imputation by chained equations was used to fill is missing data for a completed data set. (28) The variables included in the imputation model were: age, American Society of Anaesthesiologists physical status (ASA) score, NF-1, prior radiotherapy on same location, nerve type, tumour size, tumour depth, triton, grade, margins, radiotherapy, and chemotherapy (CTX). Furthermore, we included the event indicator and Nelson-Aalen estimator for the cumulative baseline hazard in the imputation model. (28) Twenty imputed datasets were created as part of the multiple imputation (m=20). Estimates from the imputed datasets were combined using Rubin's rules. For one centre, no information on grade, depth and triton was available, as these variables were not included in their database. This centre was only included as validation cohort in the internal-external cross validation procedure after imputation of the systematically missing variables. It was not used for model development.

Model performance was assessed by examining discrimination and calibration. Discrimination was measured using the concordance index (C-index). Discrimination of a time-to-event model relates to how well the model could distinguish between patients with a shorter time-to-event from patients with a longer time-to-event. A

C-index of 0.5 indicates that the model is no better than chance, whereas a C-index of 1 indicates perfect discrimination. (29) Calibration was assessed with the Observed/Expected (O/E) statistic, and visually by plotting the predicted against the observed OS at 3, 5, and 10 years. The 45 degrees line is a reference for perfect calibration. (30)

The clinical usefulness of the model was assessed by decision curve analysis (DCA). (31) Clinical guidelines recommend considering perioperative CTX in a selected group of patients based on risk-predicting tools such as the MPNST-specific model. (17) For illustrative purposes a decision threshold for treatment with perioperative CTX was set at 34%, based on literature, to calculate the net benefit, sensitivity, and specificity of the prediction tool at this threshold. (32) This threshold implies that we allow for overtreatment of approximately 2 patients (who would survive without additional treatment) per correctly treated patient (who would die without additional treatment), since a 1:2 ratio implies a probability threshold of 33%.

To provide individual predictions based on the updated model, a web-based tool was built and published on www.evidencio.com (MONACO prediction tool: Survival after resection of malignant peripheral nerve sheath tumours). An interactive tool in Excel spreadsheet is available including all estimates to validate, update, and incorporate the predictors in existing or new tools.

Baseline characteristics were described with proportions for categorical variables and medians with interquartile ranges for continuous variables. Median follow-up was assessed with the reverse Kaplan-Meier method. 5-year OS stratified for baseline characteristics was estimated using the Kaplan-Meier method. All statistical tests were two-sided with a statistical significance level set at $p \leq 0.05$. The 95% confidence interval (CI) of the O/E statistic was estimated using bootstrapping (B=1000). All statistical analyses were performed in R (version 4.1.2) with the packages 'mice', 'survival', 'boot', 'rms' and 'dcurves'. (33)

Results

Study population

A total of 507 patients with primary MPNST surgically treated with curative intent were included in this study (**Appendix C**). Among them, 168 patients (33%) had NF-1 and 39 (10%) had a triton tumour. The median follow-up was 8.7 years. Baseline characteristics for the total population are presented in **Table 1** and **Appendix D**.

Table 1. Baseline characteristics

Variable	Overall (N=507)	5-yr OS (95%CI)
Age (years)		
Median (IQR)	43 (30-57)	<44: 69 (63-75) ≥44: 60 (54-67)
Neurofibromatosis type 1		
No	336 (66.7%)	67 (62-72)
Yes	168 (33.3%)	61 (53-69)
Missing	*3*	
Location		
Central	188 (37.1%)	62 (55-70)
Extremity	266 (52.5%)	68 (62-75)
Head and neck	53 (10.5%)	59 (47-74)
Size (cm)		
Median (IQR)	7 (4-11)	<7: 75 (69-81) ≥7: 58 (51-65)
Missing	*59*	
Depth		
Deep	267 (70.4%)	61 (55-67)
Superficial	112 (29.6%)	80 (72-88)
Missing	*128*	
Triton		
No	351 (90.0%)	68 (63-73)
Yes	39 (10.0%)	54 (40-74)
Missing	*117*	
Grade (FNCLCC)		
1	66 (21.9%)	92 (85-100)
2	68 (22.6%)	71 (61-84)
3	167 (55.5%)	60 (53-69)
Missing	*206*	
Surgical margin		
R0	388 (76.5%)	68 (62-73)
R1	119 (23.5%)	54 (46-65)
Radiotherapy		
Adjuvant	169 (33.8%)	58 (51-67)
Neoadjuvant	99 (19.8%)	62 (52-74)
No radiotherapy	232 (46.4%)	72 (66-78)
Missing	*7*	
Chemotherapy		
Adjuvant	31 (6.2%)	66 (50-87)
Neoadjuvant	89 (17.8%)	64 (54-75)
No chemotherapy	379 (76.0%)	65 (60-70)
Missing	*8*	
Status		
Dead	211/507	65 (61-69)

IQR: Interquartile range, FNCLCC: Fédération Nationale des Centres de Lutte Contre Le Cancer.

Validation of Sarculator and PERSARC

A subset of 207 patients, that met all the inclusion criteria of both Sarculator for eSTS and PERSARC, was considered to assess the performance of these prediction tools in an MPNST population (**Appendix E**). **Figure 1** depicts the calibration performance (O/E-statistic) and discriminative ability (C-index) of both tools across region. The C-index was 0.60 for both Sarculator and PERSARC. The predictions by Sarculator were slightly too high (O/E-statistic 0.81, 95%CI 0.71-0.91), and near perfect for PERSARC (O/E-statistic 0.95, 95%CI 0.83-1.05). The calibration plots are presented in **Appendix F**.

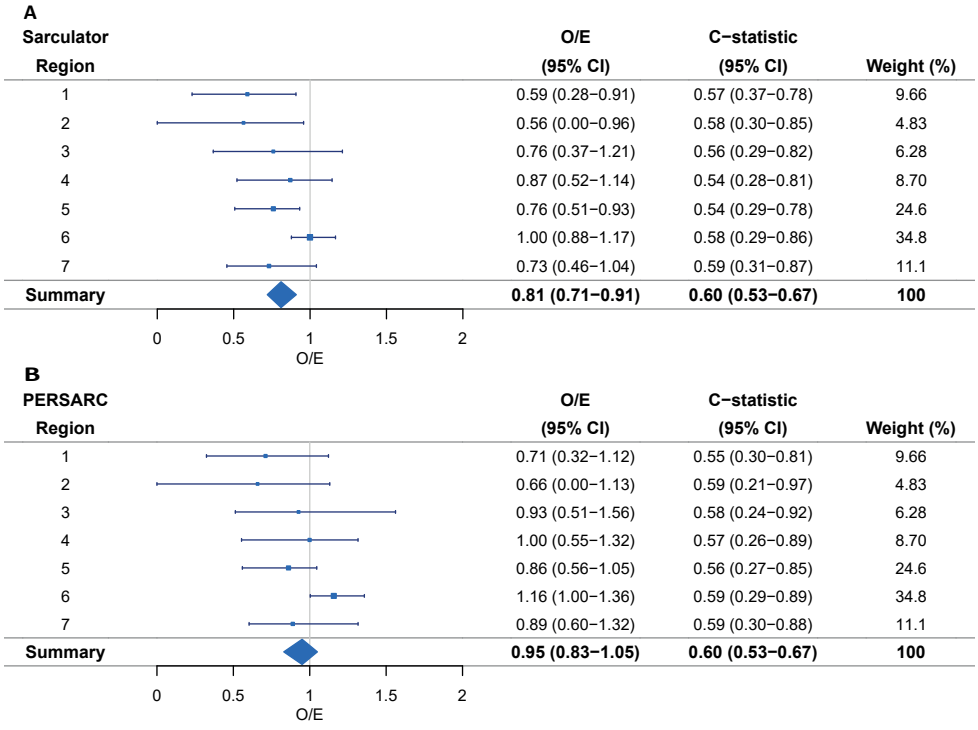


Figure 1. Calibration (O/E-statistic) and discrimination (c-statistic) of Sarculator for eSTS (A) and PERSARC (B) on different regions (see **Appendix A**).

Model extension: the MONACO tool

The final multivariable Cox model included all main effects, in which tumour size was square root transformed and age was modelled as linear variable (**Table 2**). None of the pre-specified interaction terms were statistically significant. All regression coefficients were multiplied by a shrinkage factor of 0.88 to account for overfitting in predictions. **Table 3** depicts an overview of the characteristics of the Sarculator, PERSARC and MONACO tools, respectively.

Table 2. Results of the final MONACO model before and after shrinkage (factor=0.88)

	HR (95%CI)	HR after shrinkage
Age (per 10 years)	1.29 (1.17-1.42)	1.25
Size (per √1 cm)	1.37 (1.10-1.71)	1.32
NF1		
No	1	1
Yes	1.38 (0.95-2.02)	1.33
Location		
Central	1	1
Extremity	0.83 (0.58-1.19)	0.85
Head and neck	1.69 (0.93-3.08)	1.59
Depth		
Deep	1	1
Superficial	0.49 (0.31-0.78)	0.53
Triton		
No	1	1
Yes	1.07 (0.64-1.80)	1.06
Grade		
1	1	1
2	1.63 (0.84-3.17)	1.54
3	2.71 (1.50-4.90)	2.39
Margin		
R0	1	1
R1	1.89 (1.32-2.69)	1.74

HR: hazard ratio, CI: confidence interval

Table 3. Comparison of model characteristics of Sarculator for eSTS, PERSARC and MONACO tool

Sarculator for eSTS	PERSARC	MONACO
Eligibility criteria		
18 years and older Primary (non-metastatic) STS of the extremities All FNCLCC grades (1/2/3) Surgically treated with macroscopically negative margins	18 years and older Primary (non-metastatic) STS of the extremities High grade (2/3) Surgically treated (no restrictions on surgical margins)	All ages Primary (non-metastatic) MPNST All FNCLCC grades (1/2/3) Surgically treated with macroscopically negative margins
Predictors		
Age Grade (1/2/3) Size Histology	Age Grade (2/3) Size Histology Depth Margin Radiotherapy	Age Grade (1/2/3) Size Histology Depth NF1 Triton Location
Development cohort		
Patients treated in Milan (Italy) between 1994-2013 (n=1452)	Patients treated in 5 international centres between 2001-2014 (n=766)	Patients treated in 11 international centres between 2000-2019
Nr. of patients with MPNST in development cohort		
N = 85 (6%)	N = 91 (12%)	N = 391 (100%)
Outcomes		
OS and DM (at 5 and 10 years) from definitive surgery	OS, DM and LR (at 3, 5, and 10 years) from definitive surgery	OS (at 3, 5, and 10 years) from definitive surgery

FNCLCC: Fédération Nationale des Centres de Lutte Contre le Cancer, OS: Overall survival, DM: Distant metastasis, LR: Local recurrence, NF1: Neurofibromatosis type 1

Model performance of the MONACO tool

The C-index for the final model was 0.73 (95% CI 0.69-0.77) and calibration at 5-year OS was adequate (**Figure 2**). The C-index for the seven regions ranged from 0.59 to 0.76 with a pooled C-index of 0.69 (95%CI 0.65-0.73, **Figure 3**). The model was reasonably calibrated across the regions (**Figure 3**, **Appendix G**).

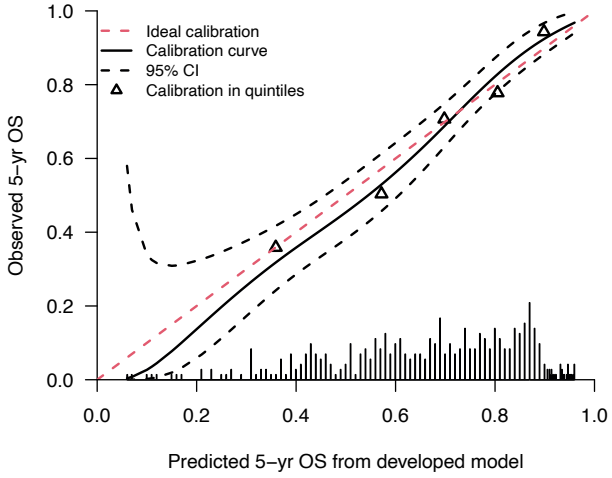


Figure 2. Calibration plot and distribution of the predictions based on the MONACO model at 5 years from definitive surgery

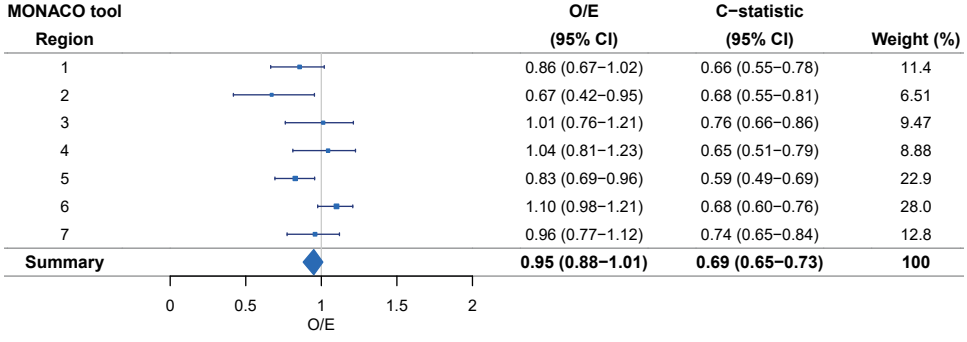


Figure 3. Discrimination (c-statistic) and calibration (O/E-statistic) of the MONACO model in an internal-external cross validation procedure across regions (see **Appendix A**)

Clinical applicability

Figure 4 depicts the decision curve of the MONACO model for the total population. This figure illustrates that using the MONACO model is clinically useful if the decision-maker – physician and/or patient –would opt for an intervention if the 5-year risk of death is ≥10%. Applying a risk-based cut-off for perioperative CTX of 34% (5-year OS ≤ 66%) results in a net benefit of 0.12 when using the MPNST-specific MONACO model. This is a higher net benefit compared with treating all or none of the patients with perioperative CTX (**Table 4**). The net benefit represents the proportion of extra true positives while accounting for false positives, meaning that 12 patients would get CTX recommended who would otherwise die within 5 years, while zero patients would receive unnecessary CTX per 100 patients. At this risk threshold the sensitivity and specificity of using the extended model were 61% (95%CI 53-68) and 73% (95%CI 68-78%), respectively.

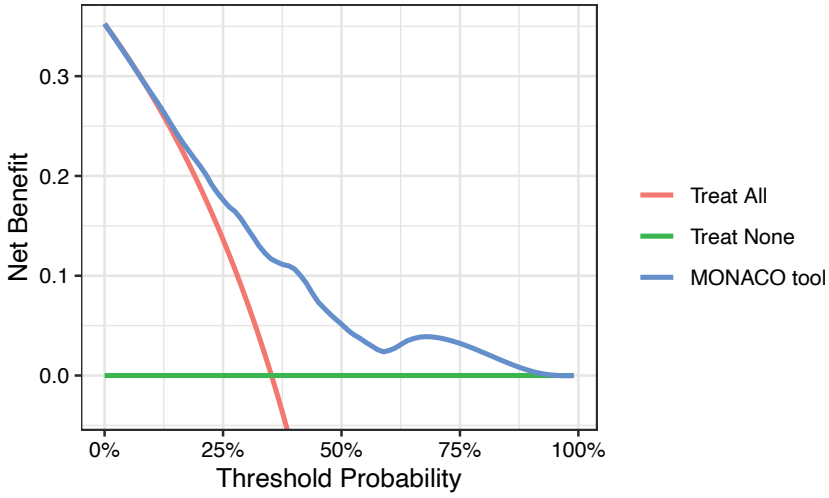


Figure 4. Decision curve analysis.

The y-axis is the net benefit, which is the sum of true positives and a weighted number of false positives. The x-axis is the preference of the patient or physician. The unit of preference is the 5-year probability of death. The lines represent the different treatment strategies: treat all patients (solid line), treating none (dotted line), or using the MONACO prediction tool to decide which patients to treat or not to treat, with the cut-off for treatment at the threshold probability (dashed line). Preference refers to how one values the harms and benefits of a certain intervention or treatment. This may vary from patient to patient or physician to physician. For example, one physician would only want to treat patients with a certain treatment, taking harms and benefits of the treatment into account, if the patients' 5-year risk of death is more than 33%. The threshold probability of physician's preference is then 33%, implying that overtreatment of 2 patients (unnecessary perioperative chemotherapy) are worth 1 necessary treatment. At this threshold probability the use of the MONACO model results in a higher net benefit that treating all or none of the patients with the certain treatment.

Table 4. Calculation of net benefit for different treatment strategies

Strategy	True positives: patients treated with CTX who would otherwise die within 5 years	False positives: patients treated with CTX who will not die within 5 years	Net benefit
Treat all with CTX	178	329	0.02
Treat none with CTX	0	0	0
Treat with CTX if 5-year mortality ≥34% according to MONACO	108	88	0.12

CTX: Perioperative chemotherapy

Discussion

The present study validated and extended existing personalised risk assessment tools for a wider range of patients with MPNSTs based on type-specific predictors. This MPNST-specific model, the MONACO tool, calculates the 3-, 5- and 10-year survival in patients with primary MPNST who underwent macroscopically complete surgical resection with curative intent. This is the first study that assessed the performance of existing generic prognostic tools in an MPNST population and updated the models with type specific predictors. All estimates have been published online to validate, update, or incorporate the estimated predictors in existing or future prediction tools.

Several prediction models have been developed for patients with primary STS. Most of the externally validated models were built for all histological types and did not include type specific predictors. (4, 5, 34, 35). In this study we assessed the performance of well-known Sarculator and PERSARC calculators in a multicentre cohort of patients with MPNST. Both had a comparable moderate discriminative ability and comparable calibration performance.

As ±50% of the MPNSTs are located outside the extremities and retroperitoneum and ±10% of the patients are younger than 18 years, the Sarculator and PERSARC tools may not be applicable for a large proportion of patients with MPNST. In addition, MPNSTs differ from other STS as they are associated with NF-1 and rhabdomyoblastic differentiation, which are common MPNST specific negative predictors for OS. (10, 12, 13, 16, 23) By extending the existing models the c-statistic improved from 0.60 for both Sarculator and PERSARC to around 0.70 at external validation. (19) Reassessment of the generic predictors and assessment of the MPNST specific predictors allowed us to further improve the ability to predict survival in patients with MPNST. However, there are several other prognostic markers that could further improve our model, while aiming for a right balance between the prognostic ability of the model and its clinical usability. A recent systematic review provided an overview

of all published prognostic molecular and immunohistochemical markers. (36) In addition, there are several international initiatives for multi-omics characterization of MPNSTs that could further improve our prognostic performance. (37) With this study we intended to initiate an MPNST specific prognostic model that could be further extended, updated, and recalibrated together with the research community. Through Evidencio (MONACO prediction tool: Survival after resection of malignant peripheral nerve sheath tumours), each institution could validate (and recalibrate) the MONACO prediction tool for its own MPNST population.

To our knowledge, only one model has previously been developed specifically for patients with MPNSTs. (38) However, this nomogram did not include MPNST-specific predictors and important generic predictors such as tumour size and grade. Furthermore, this nomogram was built based on the Surveillance, Epidemiology and End Results (SEER) database including patients with MPNST diagnosed from 1973. This is an important limitation since treatment and prognosis could be different at that time. In addition, this study included patients with distant disease at the time of presentation. (38)

Strengths and limitations

An important strength of the present study is that it is based on large cohorts of patients with MPNST including MPNST-specific predictors. The inclusion of patients from multiple centres allowed for assessment of performance across a spectrum of settings. (19) Other strengths are the easily determinable predictors included in the MONACO model. In addition to being used to obtain personalized survival probabilities and to inform patients and physicians about prognosis for shared decision-making, the MONACO tool can also be used in research settings to adjust for confounders or to assess heterogeneity in treatment effect based on prognosis. (39)

In this paper the clinical usefulness of the MONACO tool was illustrated with a decision curve, which is a relatively novel approach to performance assessment (**Figure 4**). The MONACO prediction tool can have a positive impact on decision making on perioperative CTX as illustrated for a decision threshold for perioperative CTX of 34% (5-year OS of ≤66%). (32) The decision threshold of 34% implies that the benefit of perioperative CTX for a patient who would otherwise die, is approximately worth the harm of two unnecessary treatments of patients who would survive without perioperative CTX. Obviously, the decision threshold may vary from patient to patient and from physician to physician. The MONACO tool has a positive net benefit across a wide range of possible thresholds, in particular between 25 and 60%.

This study has some limitations. One region did not record data on tumour depth, grade, and triton status. we included this region only for validation of the MONACO model. Also, longer follow-up would clarify prognosis after 5 years. Furthermore, no central pathology review was performed. Although this resembles clinical practice, we recognize that diagnosing MPNST can be challenging due to the lack of specific histologic criteria and overlapping morphologic features with other types of nerve sheath tumours. (40) Histologic evaluation sometimes require correlation with clinical and radiological findings in order to classify a tumour as MPNST. Due to these diagnostic challenges, some MPNSTs might have been misclassified. In line with improved histologic criteria and advances in (molecular) pathology in the last decades, we have restricted our inclusion period from 2000 onwards, to minimize this misclassification bias.

Finally, prediction tools should ideally be updated to improve local validity. (41) As reflected in the internal-external cross-validation, model performance differs to some extent across regions. (25) In this study, we did not yet update the model with setting-specific estimates. Through Evidencio, one could recalibrate the MONACO prediction tool for a specific population of patients with MPNST.

Conclusion

The survival of patients with primary MPNST surgically treated with curative intent can be predicted by a simple tool including MPNST-specific predictors. The MONACO tool may benefit from further validation and is applicable for a wider range of patients with MPNST compared with the existing generic STS prediction tools. All estimates have been published online to validate, update, or incorporate the estimated predictors in existing or future prediction tools.

References

1. WHO Classification of Tumours: Soft Tissue and Bone Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020.
2. Trojani M, Contesso G, Coindre JM, Rouesse J, Bui NB, de Mascarel A, et al. Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading system. Int J Cancer. 1984;33(1):37-42.
3. American Joint Committee on Cancer. AJCC cancer staging manual 8th ed. Chicago: Springer; 2017.
4. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Development and external validation of two nomograms to predict overall survival and occurrence of distant metastases in adults after surgical resection of localised soft-tissue sarcomas of the extremities: a retrospective analysis. The Lancet Oncology. 2016;17(5):671-80.
5. van Praag VM, Rueten-Budde AJ, Jeys LM, Laitinen MK, Pollock R, Aston W, et al. A prediction model for treatment decisions in high-grade extremity soft-tissue sarcomas: Personalised sarcoma care (PERSARC). European journal of cancer (Oxford, England : 1990). 2017;83:313-23.
6. Brennan MF, Antonescu CR, Moraco N, Singer S. Lessons learned from the study of 10,000 patients with soft tissue sarcoma. Ann Surg. 2014;260(3):416-21; discussion 21-2.
7. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. European journal of cancer (Oxford, England : 1990). 2013;49(3):684-95.
8. Zagars GK, Ballo MT, Pisters PW, Pollock RE, Patel SR, Benjamin RS, et al. Prognostic factors for patients with localized soft-tissue sarcoma treated with conservation surgery and radiation therapy: an analysis of 1225 patients. Cancer. 2003;97(10):2530-43.
9. Watson KL, Al Sannaa GA, Kivlin CM, Ingram DR, Landers SM, Roland CL, et al. Patterns of recurrence and survival in sporadic, neurofibromatosis Type 1-associated, and radiation-associated malignant peripheral nerve sheath tumors. J Neurosurg. 2017;126(1):319-29.
10. Miao R, Wang H, Jacobson A, Lietz AP, Choy E, Raskin KA, et al. Radiation-induced and neurofibromatosis-associated malignant peripheral nerve sheath tumors (MPNST) have worse outcomes than sporadic MPNST. Radiother Oncol. 2019;137:61-70.
11. Basu TN, Gutmann DH, Fletcher JA, Glover TW, Collins FS, Downward J. Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. Nature. 1992;356(6371):713-5.
12. Acem I, Martin E, van Houdt WJ, van de Sande MAJ, Grünhagen DJ, Verhoef C, et al. The Association of Metastasis Pattern and Management of Metastatic Disease with Oncological Outcomes in Patients with Malignant Peripheral Nerve Sheath Tumors: A Multicenter Cohort Study. Cancers (Basel). 2021;13(20).
13. Kamran SC, Howard SA, Shinagare AB, Krajewski KM, Jagannathan JP, Hornick JL, et al. Malignant peripheral nerve sheath tumors: prognostic impact of rhabdomyoblastic differentiation (malignant triton tumors), neurofibromatosis 1 status and location. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2013;39(1):46-52.
14. Voss RK, Callegaro D, Chiang YJ, Fiore M, Miceli R, Keung EZ, et al. Sarculator is a Good Model to Predict Survival in Resected Extremity and Trunk Sarcomas in US Patients. Annals of surgical oncology. 2022.
15. Gronchi A, Miceli R, Shurell E, Eilber FC, Eilber FR, Anaya DA, et al. Outcome prediction in primary resected retroperitoneal soft tissue sarcoma: histology-specific overall survival and

disease-free survival nomograms built on major sarcoma center data sets. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31(13):1649-55.
16. Martin E, Coert JH, Flucke UE, Slooff W-BM, Ho VKY, van der Graaf WT, et al. A nationwide cohort study on treatment and survival in patients with malignant peripheral nerve sheath tumours. European journal of cancer (Oxford, England : 1990). 2020;124:77-87.
17. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2021;32(11):1348-65.
18. Von Mehren M, Kane JM, Agulnik M, Bui MM, Ganjoo KN, George S, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. 2018;16(5):536-63.
19. Steyerberg EW, Harrell FE, Jr. Prediction models need appropriate internal, internal-external, and external validation. J Clin Epidemiol. 2016;69:245-7.
20. Kattan MW, Hess KR, Amin MB, Lu Y, Moons KG, Gershenwald JE, et al. American Joint Committee on Cancer acceptance criteria for inclusion of risk models for individualized prognosis in the practice of precision medicine. CA: a cancer journal for clinicians. 2016;66(5):370-4.
21. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): the TRIPOD statement. J Clin Epidemiol. 2015;68(2):134-43.
22. Anghileri M, Miceli R, Fiore M, Mariani L, Ferrari A, Mussi C, et al. Malignant peripheral nerve sheath tumors: prognostic factors and survival in a series of patients treated at a single institution. Cancer. 2006;107(5):1065-74.
23. Martin E, Coert JH, Flucke UE, Slooff W-BM, van de Sande MAJ, van Noesel MM, et al. Neurofibromatosis-associated malignant peripheral nerve sheath tumors in children have a worse prognosis: A nationwide cohort study. Pediatric blood & cancer. 2020;67(4):e28138-e.
24. Neurofibromatosis: Conference Statement. Arch Neurol. 1988;45(5):575-8.
25. Steyerberg EW. Clinical Prediction Models: A Practical Approach to Development, Validation, and Updating: Springer International Publishing; 2019.
26. Harrell FE. Regression Modeling Strategies. 1 ed. New York: Springer New York, NY; 2001. 572 p.
27. Ioannidis JP. Why most discovered true associations are inflated. Epidemiology. 2008;19(5):640-8.
28. Van Buuren S. Flexible Imputation of Missing Data. 2nd ed. New York: Chapman and Hall/CRC; 2018. 444 p.
29. Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15(4):361-87.
30. McLernon DJ, Giardiello D, Van Calster B, Wynants L, van Geloven N, van Smeden M, et al. Assessing Performance and Clinical Usefulness in Prediction Models With Survival Outcomes: Practical Guidance for Cox Proportional Hazards Models. Ann Intern Med. 2023;176(1):105-14.
31. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making. 2006;26(6):565-74.
32. Acem I, van Houdt WJ, Grünhagen DJ, van der Graaf WTA, Rueten-Budde AJ, Gelderblom H, et al. The role of perioperative chemotherapy in primary high-grade extremity soft tissue sarcoma: a risk-stratified analysis using PERSARC. European journal of cancer (Oxford, England : 1990). 2022;165:71-80.

33. R Development Core Team. R: A language and environment for statistical computing. 3.6.3 ed. Vienna, Austria: R Foundation for Statistical Computing; 2010.
34. Kattan MW, Leung DH, Brennan MF. Postoperative nomogram for 12-year sarcoma-specific death. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(3):791-6.
35. Sampo M, Tarkkanen M, Tukiainen E, Popov P, Gustafson P, Lundin M, et al. A web-based prognostic tool for extremity and trunk wall soft tissue sarcomas and its external validation. Br J Cancer. 2012;106(6):1076-82.
36. Martin E, Acem I, Grünhagen DJ, Bovée J, Verhoef C. Prognostic Significance of Immunohistochemical Markers and Genetic Alterations in Malignant Peripheral Nerve Sheath Tumors: A Systematic Review. Front Oncol. 2020;10:594069.
37. Miller DT, Cortés-Ciriano I, Pillay N, Hirbe AC, Snuderl M, Bui MM, et al. Genomics of MPNST (GeM) Consortium: Rationale and Study Design for Multi-Omic Characterization of NF1-Associated and Sporadic MPNSTs. Genes (Basel). 2020;11(4).
38. Yan P, Huang R, Hu P, Liu F, Zhu X, Hu P, et al. Nomograms for predicting the overall and cause-specific survival in patients with malignant peripheral nerve sheath tumor: a population-based study. J Neurooncol. 2019;143(3):495-503.
39. Kent DM, Paulus JK, van Klaveren D, D'Agostino R, Goodman S, Hayward R, et al. The Predictive Approaches to Treatment effect Heterogeneity (PATH) Statement. Ann Intern Med. 2020;172(1):35-45.
40. Le Guellec S, Decouvelaere A-V, Filleron T, Valo I, Charon-Barra C, Robin Y-M, et al. Malignant Peripheral Nerve Sheath Tumor Is a Challenging Diagnosis: A Systematic Pathology Review, Immunohistochemistry, and Molecular Analysis in 160 Patients From the French Sarcoma Group Database. Am J Surg Pathol. 2016;40(7):896-908.
41. Binuya MAE, Engelhardt EG, Schats W, Schmidt MK, Steyerberg EW. Methodological guidance for the evaluation and updating of clinical prediction models: a systematic review. BMC Med Res Methodol. 2022;22(1):316.

Appendix

Appendix A. List of included centres stratified by region

Region	Centre
1	Amsterdam University Medical Centre, Amsterdam, The Netherlands
	The Netherlands Cancer Institute, Amsterdam, The Netherlands
2	Diakonessenhuis, Utrecht, The Netherlands
	University Medical Centre Utrecht, Utrecht, The Netherlands
3	Erasmus Medical Centre Cancer Institute, Rotterdam, The Netherlands
4	Leiden University Medical Centre, Leiden, The Netherlands
5	Mayo Clinic Hospital, Rochester, Minnesota, United States
6	Fondazione IRCCS Instituto Nazionale dei Tumori, Milan, Italy
7	Maastricht University Medical Centre, Maastricht, The Netherlands
	Radboud University Medical Centre, Nijmegen, The Netherlands
	University Medical Centre Groningen, Groningen, The Netherlands

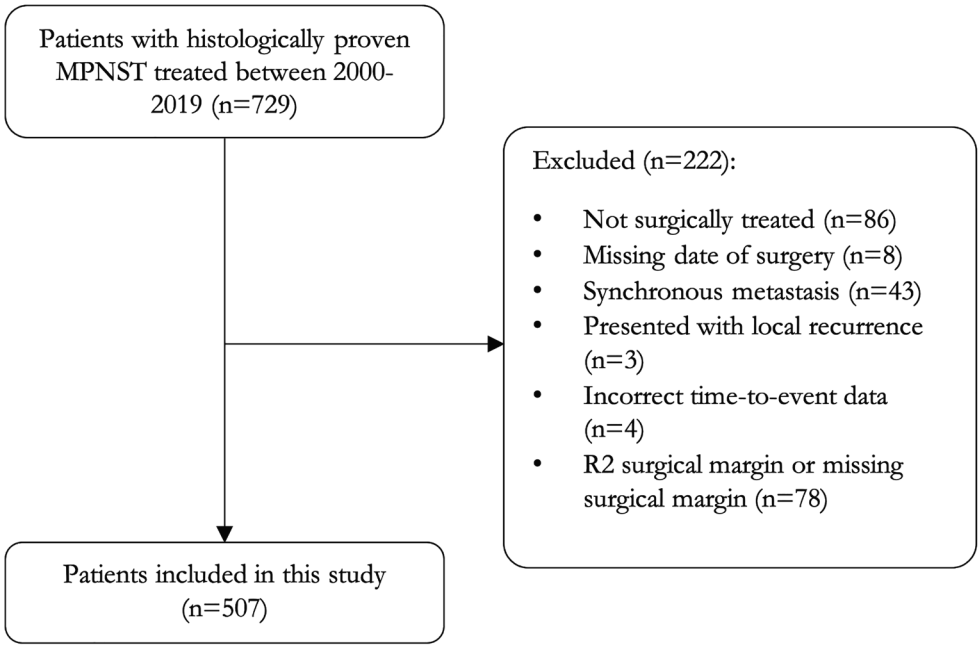
Appendix B. TRIPOD checklist

Section/Topic	Item		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	2
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	3
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	4
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	4
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	4
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	4, A1
	5b	D;V	Describe eligibility criteria for participants.	4
	5c	D;V	Give details of treatments received, if relevant.	4

Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	5
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	5
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	5
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	5
Sample size	8	D;V	Explain how the study size was arrived at.	5
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	6
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	6
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	6
	10c	V	For validation, describe how the predictions were calculated.	x
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	4-7
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	4-5
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	15
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	A2
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	7-8
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	x
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	7-9
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	7-9
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	appendix
	15b	D	Explain how to the use the prediction model.	appendix
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	12

Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	12
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	16
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	x
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	14
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	15-16
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	7
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	1

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V.



Appendix C. Study flow chart

Appendix D. Baseline characteristics stratified by region

	Region 1 (N=58)	Region 2 (N=33)	Region 3 (N=48)	Region 4 (N=45)	Region 5 (N=116)	Region 6 (N=142)	Region 7 (N=65)	Overall (N=507)
Age (years)								
Median (IQR)	42 (28-55)	46 (33-54)	46 (31-63)	51 (28-60)	39 (30-53)	43 (31-57)	42 (28-59)	43 (30-57)
NF 1								
No	47 (81.0%)	20 (60.6%)	29 (60.4%)	37 (82.2%)	72 (62.1%)	93 (65.5%)	38 (58.5%)	336 (66.3%)
Yes	10 (17.2%)	12 (36.4%)	19 (39.6%)	8 (17.8%)	44 (37.9%)	49 (34.5%)	26 (40.0%)	168 (33.1%)
Missing	1 (1.7%)	1 (3.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.5%)	3 (0.6%)
Location								
Central	19 (32.8%)	13 (39.4%)	22 (45.8%)	17 (37.8%)	39 (33.6%)	52 (36.6%)	26 (40.0%)	188 (37.1%)
Extremity	31 (53.4%)	13 (39.4%)	19 (39.6%)	25 (55.6%)	59 (50.9%)	86 (60.6%)	33 (50.8%)	266 (52.5%)
Head and neck	8 (13.8%)	7 (21.2%)	7 (14.6%)	3 (6.7%)	18 (15.5%)	4 (2.8%)	6 (9.2%)	53 (10.5%)
Size (mm)								
Median (IQR)	50 (36-108)	69 (52-83)	60 (40-100)	70 (40-109)	56 (42-90)	70 (43-90)	67 (40-95)	70 (40-105)
Missing	4 (6.9%)	1 (3.0%)	5 (10.4%)	3 (6.7%)	46 (39.7%)	0 (0%)	0 (0%)	59 (11.6%)
Depth								
Deep	35 (60.3%)	26 (78.8%)	35 (72.9%)	33 (73.3%)	0 (0%)	92 (64.8%)	46 (70.8%)	267 (52.7%)
Superficial	19 (32.8%)	7 (21.2%)	13 (27.1%)	11 (24.4%)	0 (0%)	45 (31.7%)	17 (26.2%)	112 (22.1%)
Missing	4 (6.9%)	0 (0%)	0 (0%)	1 (2.2%)	116 (100%)	5 (3.5%)	2 (3.1%)	128 (25.2%)
Triton								
No	52 (89.7%)	31 (93.9%)	45 (93.8%)	40 (88.9%)	0 (0%)	124 (87.3%)	59 (90.8%)	351 (69.2%)
Yes	5 (8.6%)	2 (6.1%)	3 (6.3%)	5 (11.1%)	0 (0%)	18 (12.7%)	6 (9.2%)	39 (7.7%)
Missing	*1 (1.7%)*	*0 (0%)*	*0 (0%)*	*0 (0%)*	*116 (100%)*	*0 (0%)*	*0 (0%)*	*117 (23.1%)*
Grade (FNCLCC)								
1	13 (22.4%)	5 (15.2%)	6 (12.5%)	9 (20.0%)	0 (0%)	19 (13.4%)	14 (21.5%)	66 (13.0%)
2	5 (8.6%)	3 (9.1%)	9 (18.8%)	13 (28.9%)	0 (0%)	34 (23.9%)	4 (6.2%)	68 (13.4%)

	Region 1 (N=58)	Region 2 (N=33)	Region 3 (N=48)	Region 4 (N=45)	Region 5 (N=116)	Region 6 (N=142)	Region 7 (N=65)	Overall (N=507)
3	16 (27.6%)	8 (24.2%)	19 (39.6%)	22 (48.9%)	0 (0%)	89 (62.7%)	13 (20.0%)	167 (32.9%)
Missing	24 (41.4%)	17 (51.5%)	14 (29.2%)	1 (2.2%)	116 (100%)	0 (0%)	34 (52.3%)	206 (40.6%)
Margin								
R0	41 (70.7%)	20 (60.6%)	25 (52.1%)	22 (48.9%)	107 (92.2%)	131 (92.3%)	42 (64.6%)	388 (76.5%)
R1	17 (29.3%)	13 (39.4%)	23 (47.9%)	23 (51.1%)	9 (7.8%)	11 (7.7%)	23 (35.4%)	119 (23.5%)
Radiotherapy								
Adjuvant	17 (29.3%)	16 (48.5%)	29 (60.4%)	23 (51.1%)	28 (24.1%)	35 (24.6%)	21 (32.3%)	169 (33.3%)
Neoadjuvant	4 (6.9%)	4 (12.1%)	3 (6.3%)	7 (15.6%)	37 (31.9%)	36 (25.4%)	8 (12.3%)	99 (19.5%)
No radiotherapy	36 (62.1%)	13 (39.4%)	14 (29.2%)	14 (31.1%)	48 (41.4%)	71 (50.0%)	36 (55.4%)	232 (45.8%)
Missing	1 (1.7%)	0 (0%)	2 (4.2%)	1 (2.2%)	3 (2.6%)	0 (0%)	0 (0%)	7 (1.4%)
Chemotherapy								
Adjuvant	2 (3.4%)	0 (0%)	4 (8.3%)	0 (0%)	13 (11.2%)	12 (8.5%)	0 (0%)	31 (6.1%)
Neoadjuvant	5 (8.6%)	1 (3.0%)	1 (2.1%)	2 (4.4%)	28 (24.1%)	51 (35.9%)	1 (1.5%)	89 (17.6%)
No chemotherapy	51 (87.9%)	32 (97.0%)	42 (87.5%)	43 (95.6%)	68 (58.6%)	79 (55.6%)	64 (98.5%)	379 (74.8%)
Missing	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	7 (6.0%)	0 (0%)	0 (0%)	8 (1.6%)
Status								
Alive	34 (58.6%)	15 (45.5%)	29 (60.4%)	26 (57.8%)	50 (43.1%)	103 (72.5%)	39 (60.0%)	296 (58.4%)
Dead	24 (41.4%)	18 (54.5%)	19 (39.6%)	19 (42.2%)	66 (56.9%)	39 (27.5%)	26 (40.0%)	211 (41.6%)
Follow-up (years)								
Median (95%CI)	9.3 (7.0-11)	7.5 (5.5-14)	9.6 (5.9-14)	8.2 (6.5-14)	11 (8.9-14)	7.8 (5.8-8.7)	7.7 (6.2-9.5)	8.7 (7.9-9.5)

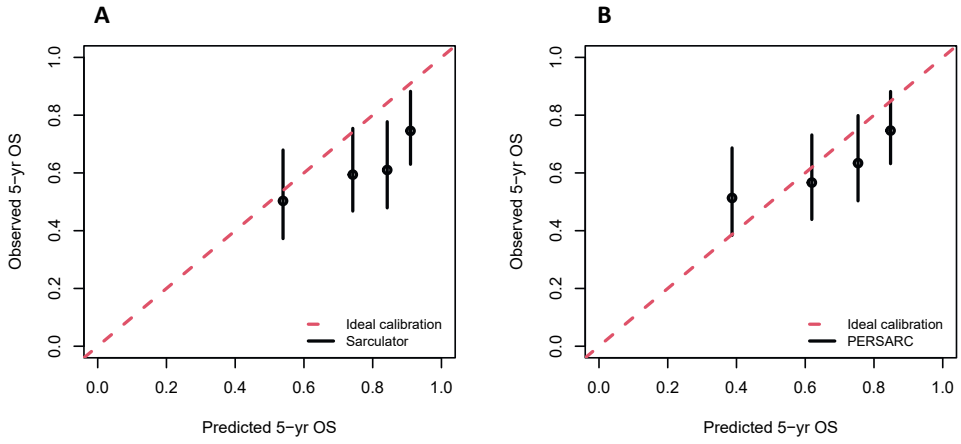
N: number of patients, FNCLCC: The Fédération Nationale des Centres de Lutte Contre le Cancer grading system, CI: confidence interval.

Appendix E. Baseline characteristics of the subpopulation of patients with MPNST of the extremities stratified by region

	Region 1 (N=20)	Region 2 (N=10)	Region 3 (N=13)	Region 4 (N=18)	Region 5 (N=51)	Region 6 (N=72)	Region 7 (N=23)	Overall (N=207)
Age (years)								
Median (IQR)	50 (39-59)	40 (25-49)	46 (43-69)	51 (30-57)	37 (31-53)	44 (31-57)	50 (30-62)	44 (31-57)
NF 1								
No	17 (85.0%)	5 (50.0%)	10 (76.9%)	15 (83.3%)	30 (58.8%)	43 (59.7%)	14 (60.9%)	134 (64.7%)
Yes	3 (15.0%)	4 (40.0%)	3 (23.1%)	3 (16.7%)	21 (41.2%)	29 (40.3%)	9 (39.1%)	72 (34.8%)
Missing	0 (0%)	1 (10.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
Size (mm)								
Median (IQR)	8.0 (4.0-13)	6.5 (5.5-7.0)	6.0 (5.5-13)	6.5 (4.2-9.8)	6.0 (4.4-9.6)	9.0 (5.0-12)	7.8 (4.7-12)	7.0 (4.3-12)
Missing	1 (5.0%)	1 (10.0%)	1 (7.7%)	0 (0%)	17 (33.3%)	0 (0%)	0 (0%)	20 (9.7%)
Depth								
Deep	10 (50.0%)	9 (90.0%)	11 (84.6%)	14 (77.8%)	0 (0%)	53 (73.6%)	21 (91.3%)	118 (57.0%)
Superficial	8 (40.0%)	1 (10.0%)	2 (15.4%)	4 (22.2%)	0 (0%)	19 (26.4%)	1 (4.3%)	35 (16.9%)
Missing	2 (10.0%)	0 (0%)	0 (0%)	0 (0%)	51 (100%)	0 (0%)	1 (4.3%)	54 (26.1%)
Triton								
No	19 (95.0%)	10 (100%)	11 (84.6%)	18 (100%)	0 (0%)	62 (86.1%)	21 (91.3%)	141 (68.1%)
Yes	1 (5.0%)	0 (0%)	2 (15.4%)	0 (0%)	0 (0%)	10 (13.9%)	2 (8.7%)	15 (7.2%)
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)	51 (100%)	0 (0%)	0 (0%)	51 (24.6%)
Grade (FNCLCC)								
1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
2	1 (5.0%)	0 (0%)	3 (23.1%)	7 (38.9%)	0 (0%)	19 (26.4%)	1 (4.3%)	31 (15.0%)
3	6 (30.0%)	1 (10.0%)	8 (61.5%)	11 (61.1%)	0 (0%)	53 (73.6%)	6 (26.1%)	85 (41.1%)
Missing	*13 (65.0%)*	*9 (90.0%)*	*2 (15.4%)*	*0 (0%)*	*51 (100%)*	*0 (0%)*	*16 (69.6%)*	*91 (44.0%)*
Margin								
R0	14 (70.0%)	6 (60.0%)	8 (61.5%)	10 (55.6%)	47 (92.2%)	67 (93.1%)	13 (56.5%)	165 (79.7%)

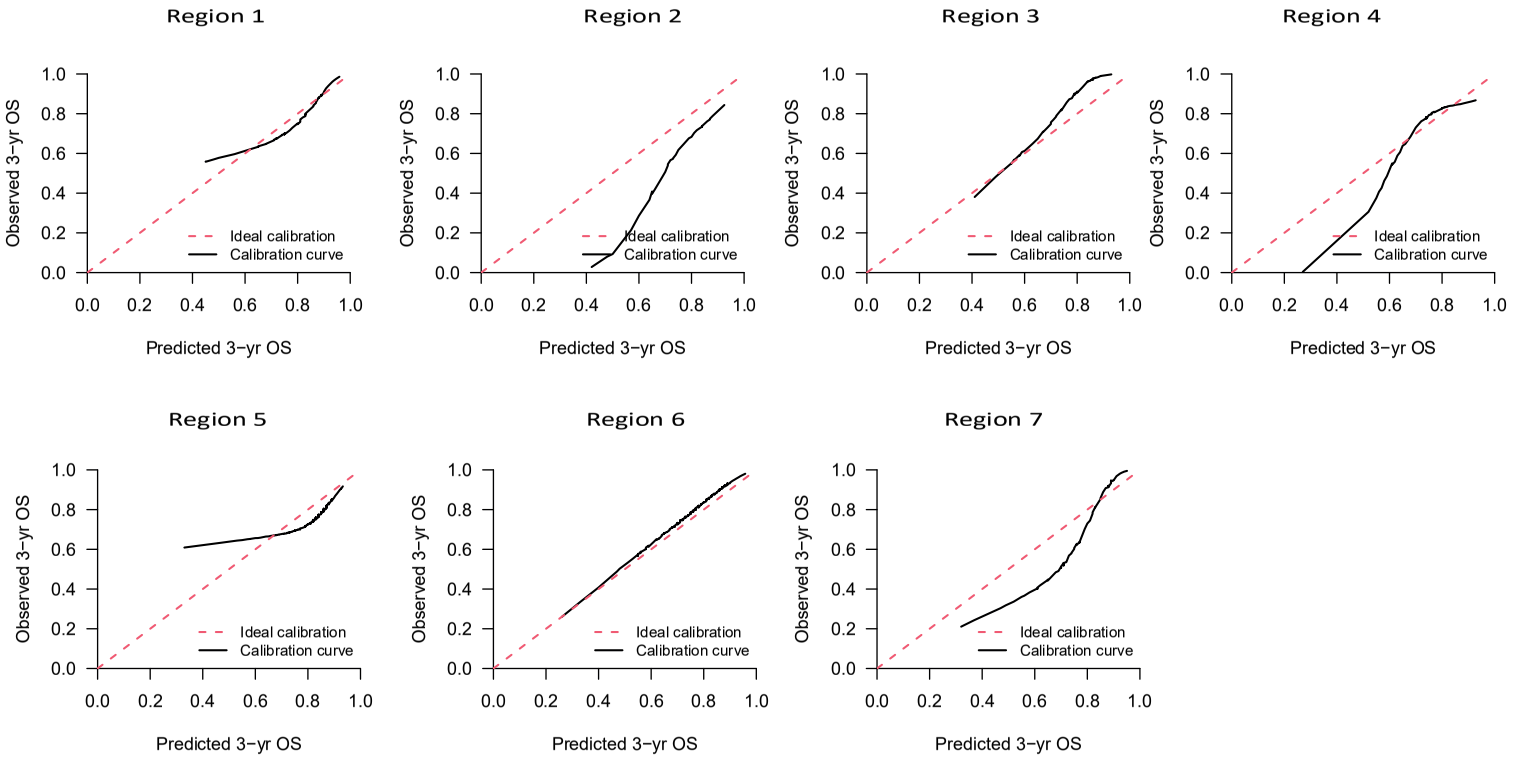
	Region 1 (N=20)	Region 2 (N=10)	Region 3 (N=13)	Region 4 (N=18)	Region 5 (N=51)	Region 6 (N=72)	Region 7 (N=23)	Overall (N=207)
R1	6 (30.0%)	4 (40.0%)	5 (38.5%)	8 (44.4%)	4 (7.8%)	5 (6.9%)	10 (43.5%)	42 (20.3%)
Radiotherapy								
Adjuvant	9 (45.0%)	5 (50.0%)	10 (76.9%)	11 (61.1%)	15 (29.4%)	25 (34.7%)	14 (60.9%)	89 (43.0%)
Neoadjuvant	3 (15.0%)	2 (20.0%)	2 (15.4%)	3 (16.7%)	23 (45.1%)	21 (29.2%)	1 (4.3%)	55 (26.6%)
No radiotherapy	8 (40.0%)	3 (30.0%)	1 (7.7%)	3 (16.7%)	13 (25.5%)	26 (36.1%)	8 (34.8%)	62 (30.0%)
Missing	0 (0%)	0 (0%)	0 (0%)	1 (5.6%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
Chemotherapy								
Adjuvant	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (11.8%)	8 (11.1%)	0 (0%)	14 (6.8%)
Neoadjuvant	1 (5.0%)	0 (0%)	0 (0%)	0 (0%)	14 (27.5%)	26 (36.1%)	1 (4.3%)	42 (20.3%)
No chemotherapy	19 (95.0%)	10 (100%)	13 (100%)	18 (100%)	26 (51.0%)	38 (52.8%)	22 (95.7%)	146 (70.5%)
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (9.8%)	0 (0%)	0 (0%)	5 (2.4%)
Status								
Alive	10 (50.0%)	5 (50.0%)	6 (46.2%)	9 (50.0%)	23 (45.1%)	53 (73.6%)	9 (39.1%)	115 (55.6%)
Dead	10 (50.0%)	5 (50.0%)	7 (53.8%)	9 (50.0%)	28 (54.9%)	19 (26.4%)	14 (60.9%)	92 (44.4%)

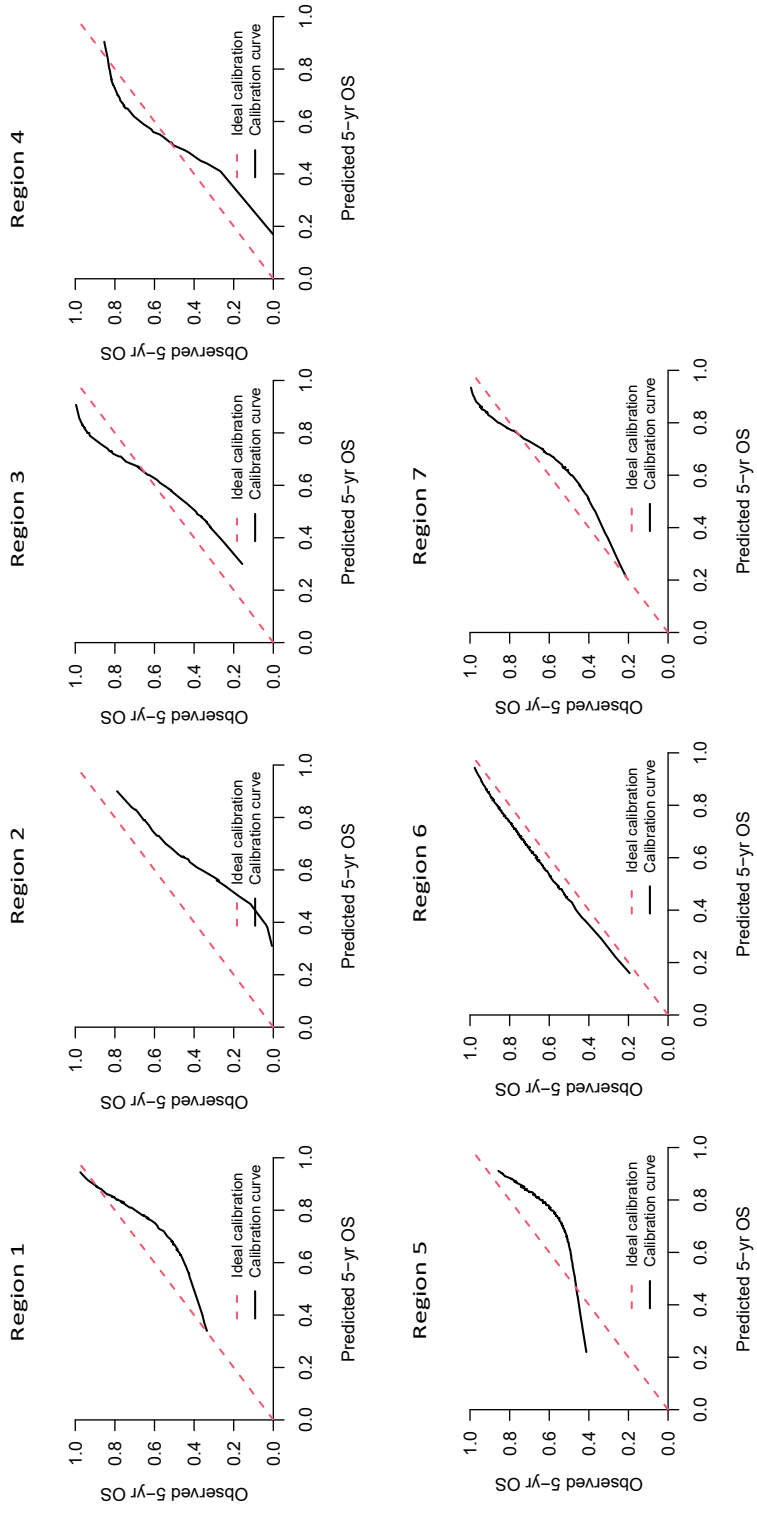
N: number of patients, FNCLCC: The Fédération Nationale des Centres de Lutte Contre le Cancer grading system, CI: confidence interval.

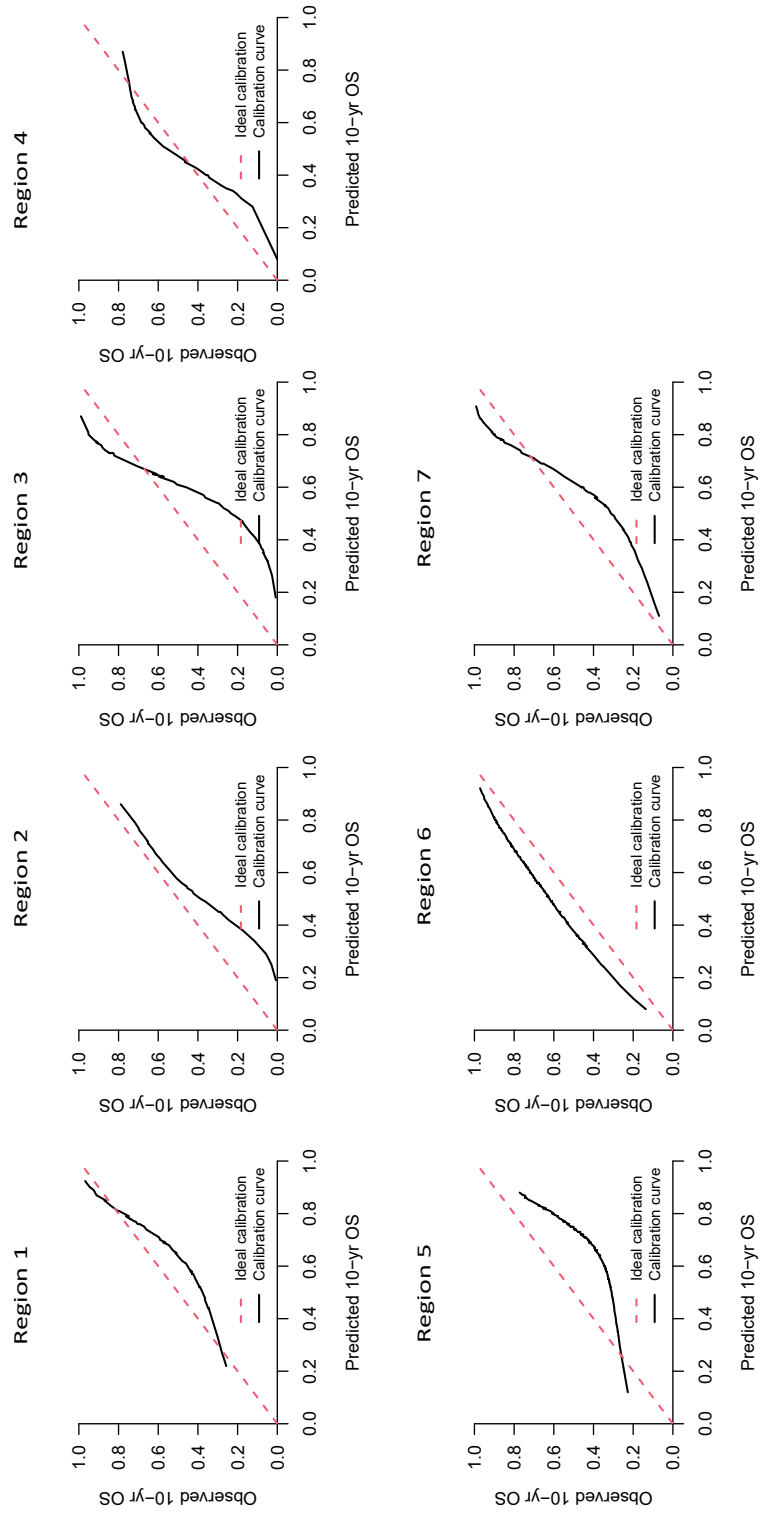


Appendix F. Calibration plots of Sarcultor (A) and PERSARC (B)

Appendix G. Calibration plots at 3, 5, and 10 years for each region after cross-validation




Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Observed 5−yr OS
Predicted 5−yr OS
Ideal calibration
Calibration curve
0.0
0.2
0.4
0.6
0.8
1.0


Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Observed 10−yr OS
Predicted 10−yr OS
Ideal calibration
Calibration curve
0.0
0.2
0.4
0.6
0.8
1.0

PART III

The Management of Sarcoma: From One-Size-Fits-All to Patient-Tailored Medicine

Chapter 8

Management of Soft Tissue Sarcomas in Extremities: Variation in Treatment Recommendations and Surveillance According to Specialty and Continent

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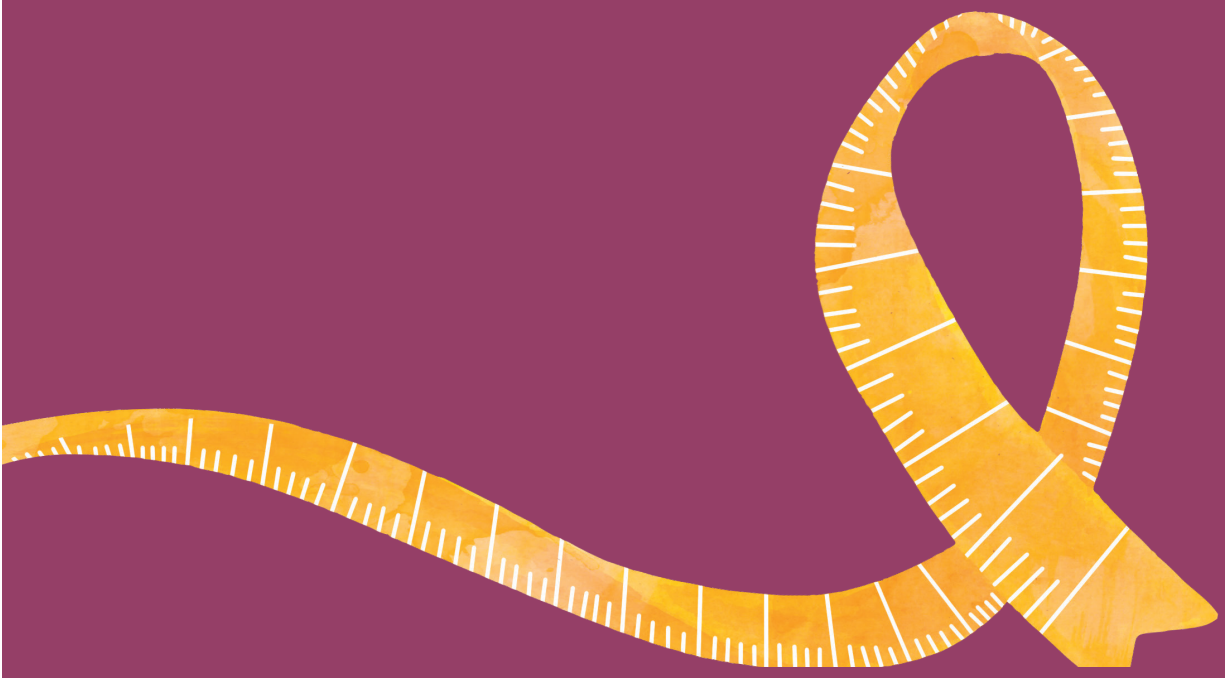


Abstract

Background

This study aimed to provide an insight into clinical decision-making and surveillance strategy of sarcoma specialists for patients with primary soft tissue sarcoma of the extremities (eSTS). The secondary aim was to quantify the role of patient- and tumour-specific factors in the perioperative management.

Methods

Members of sarcoma societies were sent a web-based 21-item survey about eSTS management. The survey concerned only primary resectable high-grade eSTS in adults.

Results

The study enrolled 396 respondents. The majority of the surgical specialists thought the evidence for perioperative chemotherapy (CTX) for high-grade eSTS was insufficient. Radiotherapy (RTX) was less frequently offered in Asia than in North America and Europe. The specialties and continents also differed regarding the importance of patient and tumour characteristics influencing RTX and CTX recommendation. For surveillance after initial treatment outpatient visits, chest computed tomography (CT) scans, and magnetic resonance images of the extremity were the methods primarily used. The specialists in North America preferred chest CT scan over chest x-ray, whereas those in Asia and Europe had no clear preference.

Conclusion

Specialty and continent are important factors contributing to the variation in clinical practice, treatment recommendations, and surveillance of patients with primary resectable high-grade eSTS.

Introduction

Soft tissue sarcomas (STSs) are a heterogeneous group of tumours with a mesenchymal origin. This group of malignant tumours has more than 80 histologic subtypes and accounts for 1% of all adult malignancies. (1) STSs are rare with an estimated incidence of around 5 patients per 100,000 persons in Europe every year. (2, 3) All this together makes it challenging to generate high level evidence for the management of primary STS.

The National Comprehensive Cancer Network guideline (NCCN) (4) and the European Society of Medical Oncology guideline (ESMO) (5) are two broadly used international clinical practice guidelines for the management and surveillance of STS. The two guidelines are similar and agree that surgery is the cornerstone for the treatment of soft tissue sarcoma of the extremities (eSTS). (4, 5) Perioperative radiotherapy (RTX) is recommended to improve local control in settings wherein adequate margins are not possible or for high-grade, deep-seated tumours, or tumours 5 cm in size or larger. (4, 5) Perioperative chemotherapy (CTX) is not standard practice, but it can be offered as an option to high-risk patients after shared decision making. (4, 5)

Although several studies have shown that adherence to guidelines results in better patient outcomes, 32–70% of patients with STS are not consistently treated in accordance with the clinical guidelines. (6-11) This study aimed to acquire insight into the variation of eSTS management by assessing the influence of clinical specialty and continent on clinical practice and surveillance. Additionally, this study investigated the extent to which selected patient and disease characteristics are used to distinguish between high- and low-risk patients and the extent to which these factors are used in clinical decision-making for perioperative treatment.

Methods

Survey design

The survey used for this study was developed by the authors after literature review and a small focus group discussion. Pilot testing of the survey was performed internally for content and face validity at Leiden University Medical Centre, The Netherlands Cancer Institute and Erasmus MC Cancer Institute in The Netherlands. Online survey software (Qualtrics; Provo, UT, USA) was used to administer the survey, which was open to respondents for a 4-month period from 2 March to 2 July 2020.

The study population received an invitation e-mail from the participating sarcoma societies describing the purpose of the survey and containing an electronic link to the online survey software. The study population received two new invitations in a time frame of 4 months as reminder. An opt-out option was provided in the request e-mail.

The survey included questions pertaining respondent characteristics, the current clinical practice, the importance of selected patient and disease characteristics in the recommendation of perioperative treatment, and follow-up evaluation. Most questions required scoring of characteristics on a 5-point Likert scale. The survey was designed with closed-ended questions only to allow a completion time of approximately 10 minutes. The respondents were allowed to leave a question blank.

The 21-items survey is available in **Appendix A**. The questions in the survey concerned only primary eSTS in adults (age ≥18 years). Additional treatment with isolated limb perfusion, immunotherapy and regional hyperthermia are not considered in this survey.

Survey responses were anonymously collected and no information that could potentially identify a respondent was collected. This study was approved by the institutional Medical Ethical Committee Leiden-Den Haag-Delft (N20.016) and complied with the regulations governing Good Clinical Research Practice and General Data Protection Regulation.

Study population

The target group for the questionnaire comprised clinically active international members of the Connective Tissue Oncology Society (CTOS), the European Musculo-Skeletal Oncology Society (EMSOS), and the Asia Pacific Musculoskeletal Tumour Society (APMSTS). Respondents who were not physicians or did not have a self-declared interest in STS were excluded from the study.

Real-world data

Findings on perioperative treatment in eSTS were compared with real-world data of 6265 patients with surgically treated primary high-grade eSTS (age ≥18 years) from 21 sarcoma centres. Details on this retrospective cohort were reported by Acem et al. (12)

Statistical analysis

All analyses were performed in the statistical program R (R Core Team, Vienna, Austria). (13) Respondent characteristics and other categorical variables are described

in absolute values and proportions. 5-point Likert scale scores were displayed in proportions and means (mean 5-pt LSS) with standard deviations (SD).

All the questions were stratified by specialty and continent. The respondents with a specialty in both medical and radiation oncology (clinical oncology) were classified as medical oncologist. Respondents from Africa, Central and South America, Australia, New Zealand, Oceania were excluded from the analyses that were stratified by continent due to insufficiently large sample sizes.

Differences in outcomes on the 5-point LSS were tested with the One-way analysis of variance (ANOVA) test. Differences in categorical outcomes were tested with the chi-square test or Fisher's exact test when the value of at least one cell in the contingency table was below 5. Bonferroni correction was used to account for multiple testing. Blank questions were considered missing and were not imputed.

Results

Demographics

The survey was received by 1386 potential respondents and completed by 428 respondents (response rate 30.9%), of whom 396 met the inclusion criteria. The study excluded respondents without a special interest in STS (n = 31) and respondents who were not physicians (n = 1). The last question of the survey was answered by 255 respondents (64.4%). **Appendix B** presents a flowchart of the respondent inclusion.

The baseline characteristics of the respondents are depicted in **Table 1**. Most of the respondents were orthopaedic oncologists (43.2%, n=171), practiced in Europe (44.9%, n=155), and had more than 15 years of experience after fellowship (36.9%, n=146).

Table 1. Baseline characteristics of the respondents

Characteristics	Overall (N=396)
Specialty	
Medical oncology	89 (22.5%)
Orthopaedic oncology	171 (43.2%)
Radiation oncology	28 (7.1%)
Surgical oncology	83 (21.0%)
Other [a]	25 (6.3%)
Years since completion of fellowship	
I am a fellow in-training	15 (3.8%)
<5 years	73 (18.5%)
5-10 years	90 (22.8%)
11-15 years	65 (16.5%)
≥15 years	151 (38.3%)
Missing	2
Current practice location	
Africa	3 (0.8%)
Asia	83 (21.0%)
Australia/New Zealand/Oceania	16 (4.0%)
Central/South America	7 (1.8%)
Europe	155 (39.1%)
North America	132 (33.3%)
Number of new cases annually	
<5	28 (7.1%)
5-25	95 (24.0%)
25-50	92 (23.2%)
≥50	181 (45.7%)

[a] Including paediatric and adolescent oncology and pathology.

Distinction between high- and low-risk patients

The characteristics primarily used to distinguish between high- and low-risk eSTS patients were grade (mean 5-pt LSS, 4.93), histologic subtype (mean 5-pt LSS, 4.65), and size (mean 5-pt LSS, 4.51) (**Figure 1**). Gender (mean 5-pt LSS, 1.52) and age (mean 5-pt LSS, 2.66) were the least important factors used to distinguish between high- and low-risk eSTS patients.

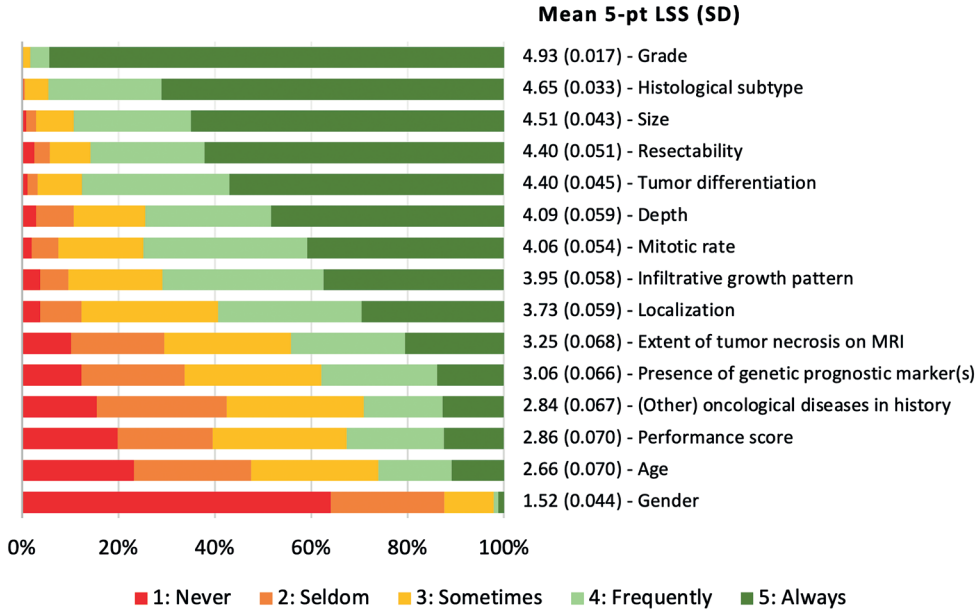


Figure 1. The use of patient and disease characteristics to distinguish between high-risk and low-risk eSTS patients (n=348)

5-pt LSS:5-point Likert Scale Score. SD: standard deviation

For surgical specialties, extent of tumour necrosis on MRI (mean 5-pt LSS, 3.51) and infiltrative growth pattern (mean 5-pt LSS, 4.12) were more important for distinguishing between high- and low-risk patients than non-surgical specialties (mean 5-pt LSS, 3.51 vs 2.84 [$p < 0.001$] and 4.12 vs 3.56 [$p < 0.001$], respectively). For non-surgical specialties, size (mean 5-pt LSS, 4.75) was more important for distinguishing between high- and low-risk patients than surgical specialties (mean 5-pt LSS, 4.40; $p < 0.001$). The use of patient and disease characteristics stratified by specialty are depicted in **Appendix C**.

To distinguish between high- and low-risk patients, the specialists in Asia and Europe gave a higher rating of importance than the specialists in North America for extent of tumour necrosis on MRI (mean 5-pt LSS, 3.75 vs. 2.80 [$p < 0.001$] and 3.43 vs 2.80 [$p < 0.001$], respectively) and infiltrative growth pattern (mean 5-pt LSS, 4.21 vs 3.71 [$p < 0.001$] and 4.11 vs 3.71 [$p = 0.004$], respectively).

Current practice of RTX in the management of high-grade eSTS

Of the 301 respondents, 142 (47.2%) treated their high-risk eSTS patients frequently (≥75%) with perioperative RTX. In Asia, RTX was offered less often (17.5%) than in Europe (52.1%; $p < 0.001$) or North America (62.4%; $p < 0.001$) (**Figure 2A**).

This was in accordance with the real-world data showing that 19.6% of the patients received RTX in Asia compared with 62.2% in Europe ($p < 0.001$) and 74.3% in Europe and North America ($p < 0.001$) (**Appendix D**).

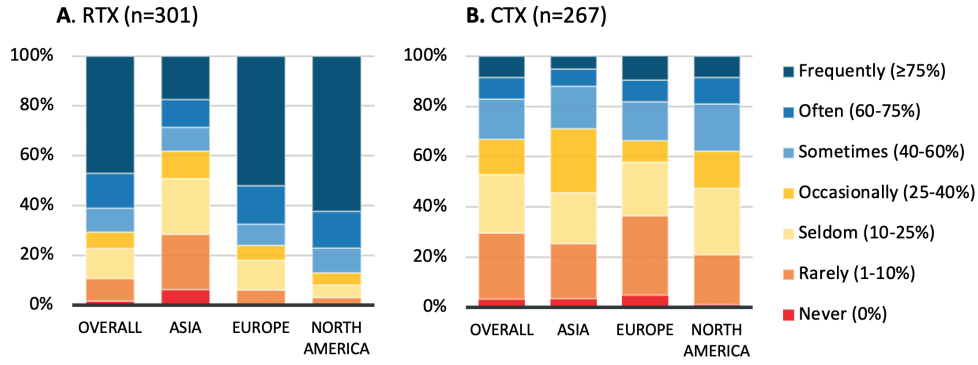


Figure 2. What percentage of your high-grade eSTS patients receive perioperative treatment? **A.** Radiotherapy. **B.** Chemotherapy

Factors influencing RTX recommendation

The factors most likely to influence perioperative RTX recommendation were the margins achieved (mean 5-pt LSS, 4.58), the anticipated margins (mean 5-pt LSS, 4.63), and grade (mean 5-pt LSS, 4.59) (**Figure 3**). The least important factors influencing RTX recommendation were gender (mean 5-pt LSS, 1.38) and presence of a genetic prognostic marker or markers (mean 5-pt LSS, 2.44).

For surgical specialties, infiltrative growth pattern was a more important factor influencing CRTX recommendation (mean 5-pt LSS, 4.13) than nonsurgical specialties (mean 5-pt LSS, 3.51; $p < 0.001$). For nonsurgical specialties, grade (mean 5-pt LSS, 4.74), performance score (mean5-pt LSS, 3.33), and oncologic history (mean 5-pt LSS, 2.82) were more important factors influencing CRTX recommendation than surgical specialties (mean 5-pt LSS: 4.52 [$p = 0.025$], 2.87 [$p = 0.005$], and 2.35 [$p = 0.004$], respectively). The use of patient and disease characteristics for RTX recommendation stratified by specialty are depicted in **Appendix E**.

The specialists in Europe and North America rated grade for recommendation of perioperative RTX as more important than did the specialists in Asia (mean 5-pt LSS, 4.69 vs 4.22 [$p = 0.003$] and 4.83 vs 4.22 [$p < 0.001$], respectively). The specialists in North America rated size for the recommendation of perioperative RTX as more important than did the specialists in Asia and Europe (mean 5-pt LSS, 4.59 vs. 4.12 [$p = 0.001$] and 4.59 vs 4.29 [$p = 0.020$], respectively).

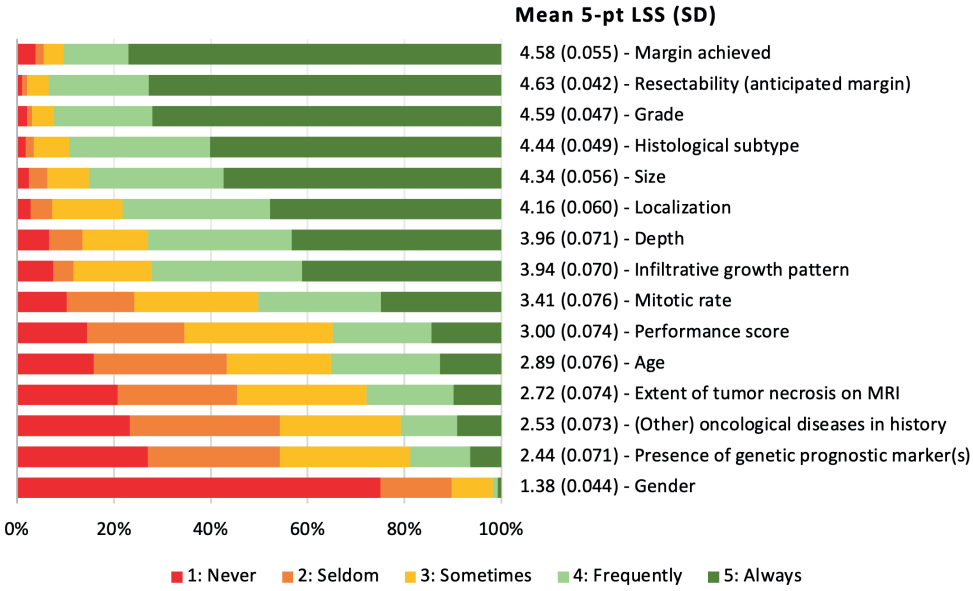


Figure 3. Factors influencing RTX recommendation (n=291)
5-pt LSS:5-point Likert Scale score. SD: standard deviation

Use of a prediction tool for RTX recommendation

Of the 296 respondents 219 (74%) would consider using a prediction tool to indicate perioperative RTX for eSTS patients. Surgical oncologists (92.2%) would consider using a prediction tool more often than orthopaedic oncologists (65.7%; $p < 0.001$). Specialists in Asia were less likely to consider using a prediction tool (50%) than specialists in Europe (76.1%; $p < 0.001$) or North America (84.2%; $p < 0.001$).

Current practice of CTX in the management of high-grade eSTS

Of the 276 respondents, 194 (70.3%) treated more than 10% of their high-risk eSTS patients with perioperative CTX (**Figure 2B**). No significant differences were found among continents in the use of CTX for high-grade eSTS. However, the real-world data showed a significant difference in the use of CTX among continents. In Asia, CTX was administered to 30.6% of the patients, whereas perioperative CTX was administered to 12.6% of the patients in Europe ($p < 0.001$) and to 3.3% of the patients North America ($p < 0.001$) (**Appendix D**)

Of the 276 respondents, 173 (62.7%) did not think the evidence was sufficient to use of perioperative CTX for patients with primary high-grade eSTS. The majority of the orthopaedic (74%) and surgical (73.3%) oncologists ($p < 0.001$) considered the current level of evidence for the role of CTX in high-grade eSTS to be insufficient, compared with 35.7% of the medical oncologists ($p < 0.001$). The attitude toward the

role of perioperative CTX in primary high-grade eSTS did not differ across continents (p = 0.137).

Older age (≥ 70 years) was thought by 120 (43%) of the 278 respondents to be an absolute contraindication for perioperative CTX.

Factors influencing CTX recommendation

The factors most likely to influence perioperative CTX recommendation were histological subtype (mean 5-pt LSS 4.73), grade (mean 5-pt LSS 4.55), and size (mean 5-pt LSS 4.20) (**Figure 5**). Gender (mean 5-pt LSS 1.40) and extent of tumour necrosis on MRI (mean 5-pt LSS 2.81) were the least important factors influencing CTX recommendation (**Figure 4A**).

For non-surgical specialties depth (mean 5-pt LSS: 3.92), location (mean 5-pt LSS: 3.62), performance score (mean 5-pt LSS: 4.36), and size (mean 5-pt LSS: 4.57) were more important factors influencing CTX recommendation compared with surgical specialties (mean 5-pt LSS: 3.41, p=0.004; 3.20, p=0.020; 3.64, p<0.001; 3.98, p<0.001, respectively). The use of patient and disease characteristics for CTX recommendation stratified by specialty are depicted in **Appendix F**.

The specialists in Asia and Europe compared with the specialists in North America gave a higher rate of importance to extent of tumour necrosis on MRI (mean 5-pt LSS, 3.13 vs 2.30 [p < 0.001] and 3.08 vs 2.30 [p < 0.001], respectively) and infiltrative growth pattern (mean 5-pt LSS, 3.36 vs 2.76 [p = 0.005] and 3.30 vs. 2.76 [p = 0.003], respectively) for a perioperative CTX recommendation.

The respondents would consider perioperative CTX primarily for synovial sarcoma (mean 5-pt LSS, 4.13), rhabdomyosarcoma (mean 5-pt LSS, 4.05), and myxoid liposarcoma with a round cell component (mean 5-pt LSS, 3.52). Perioperative CTX would be considered the least for fibrosarcoma (mean 5-pt LSS, 2.55) and myxofibrosarcoma (mean 5-pt LSS, 2.61) (**Figure 4B**).

Figure 4A. Factors influencing CTX recommendation. **4B.** For which histological subtypes would you generally consider perioperative CTX?

5-pt LSS:5-point Likert Scale score, SD: standard deviation, MPNST: Malignant Peripheral Nerve Sheath Tumour.

Use of a prediction tool for CTX recommendation

Of the 277 respondents, 224 (80.9%) would consider using a prediction tool to indicate perioperative CTX for eSTS patients. The specialists did not differ significantly in their attitude toward using a prediction tool for CTXs. The surgical oncologists

(92.2%) would consider using a prediction tool more often than the orthopaedic oncologists (65.7%; p < 0.001). The specialists in Asia were less likely to consider using a prediction tool (62.7%) than the specialists in Europe (82.9%; p = 0.007) or North America (88.4%; p < 0.001).

Follow-up evaluation

Outpatient visits, chest CT scan, and MRI of the extremity were the most common methods for follow-up evaluation. The frequency of each method declined with time (**Table 2**). Specialists in North America preferred chest CT scan over chest x-ray with a median number of chest CT scan of 4 times (mean 3.33) in the first year compared with no chest x-rays (mean 0.860) (p<0.001). After the first year, chest CT scan remained the preferred method in North America. Neither of the two methods were clearly preferred by specialists in Asia (median for CT vs. x-ray in the first year, 2 vs. 3; p = 0.276) or Europe (median for CT vs. x-ray in the first year, 2 vs. 2; p = 0.520). In the first 5 years of surveillance, 29% of the respondents never used chest x-ray, and 12% of the respondents never used chest CT scan. The outpatient clinic visit sequence used primarily in the first 5 years was 4-4-2-2-2 (16.9%; 42 of 248).

Table 2. follow-up schedule per year after initial treatment for high-grade eSTS (n=252)

	Mean frequency per year (median)				
Modality	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Outpatient visit	3.94 (4)	3.47 (4)	2.66 (2)	2.43 (2)	2.29 (2)
X-chest	1.89 (2)	1.82 (2)	1.49 (1)	1.39 (1)	1.27 (1)
CT-chest	2.65 (3)	2.48 (3)	1.95 (2)	1.70 (1)	1.48 (1)
X-extremity	1.17 (0)	0.968 (0)	0.807 (0)	0.892 (0)	0.743 (0)
CT-extremity	0.565 (0)	0.591 (0)	0.489 (0)	0.525 (0)	0.397 (0)
MRI-extremity	2.55 (3)	2.43 (2)	1.94 (2)	1.86 (1)	1.65 (1)
PET-CT scan	0.667 (0)	0.510 (0)	0.384 (0)	0.358 (0)	0.476 (0)

Most of the respondents (56.9%) felt comfortable to end the surveillance in patients with primary high-grade eSTS after 9-10 years of follow-up evaluation. Whereas 8.6% would follow their patients for more than 16 years or for the whole lifetime, 26% of the respondents ended the surveillance after 5-6 years (**Figure 5**).

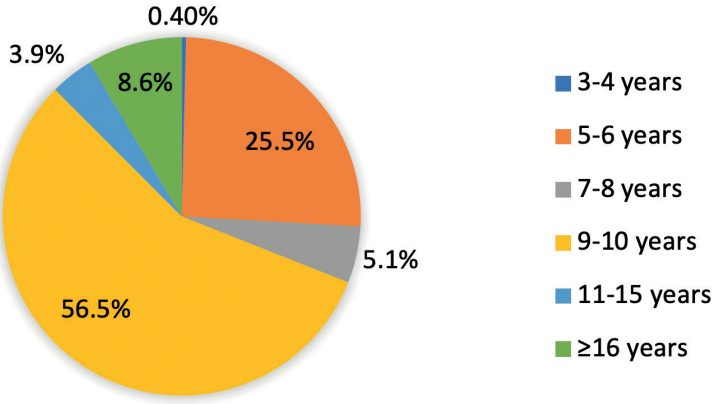


Figure 5. Duration of follow-up after primary treatment (n= 255)

Discussion

This study aimed to provide an insight into variation in the clinical decision-making processes between specialties and continents for the treatment of resectable high-grade eSTS. In addition, it aimed to analyse the relative role of specific tumour and patient factors in the clinical decision-making with regard to the perioperative treatment of these patients. This study illustrates a wide variation among specialties and continents regarding the management and surveillance of patients with eSTS. Also, the results indicate a variation in risk factors considered to be indications for perioperative treatment. However, consensus exists regarding the risk factors frequently leading to recommendation for RTX (margins, grade, histologic subtype, size) and CTX (size, histologic subtype, grade).

This study demonstrated a notable difference in RTX practice among continents, in accordance with the included real-world data. (12) In Europe and North America, most of the respondents treat 75% or more of their patients with high-grade resectable eSTS using perioperative RTX, compared with only 17.5% of the respondents in Asia. Also, we observed a greater variation of RTX use in Asia than in Europe and North America. These results are supported by a systematic review including 24 studies of the Asia-Pacific region in which the use of RTX ranged from 1 to 100% preoperatively and from 6 to 88% postoperatively. (14) The on-average lower rates of RTX use and greater variation in RTX use in Asia might be explained by a generally lower accessibility to radiotherapy in certain Asia-Pacific regions. (15)

The survey did not demonstrate a difference in CTX practice among continents. However, a notable difference in CTX use among the continents was observed in the real-world data, with CTX administration more prevalent in Asia than in Europe and North America. (12) However, the real-world data included only one high-volume centre from North America and only Japanese centres from Asia. (12)

The attitude toward the role of CTX in the management of eSTS varies widely. More than 70% of the orthopaedic and surgical oncologists did not think the evidence is sufficient for CTX in primary high-grade resectable eSTS, compared with 35% of the medical oncologists. Substantial variation also exists in the current practice of perioperative chemotherapy, with 30% of the respondents never or rarely using CTX, but with almost half of the respondents (47%) using perioperative CTX for more than 25% of their patients with primary high-grade eSTS. The variation in CTX practice might reflect a difference in interpretation of the available evidence on the role of perioperative CTX in primary eSTS. Other factors that might explain the variation are the availability of perioperative treatment and the variety of compensation and health care systems.

Several studies have suggested that a selected group of high-risk patients might benefit from perioperative CTX. (16, 17) However, the identification of these high-risk patients remains challenging. Our study demonstrated that the most important factors physicians use to identify high-risk patients are grade, histological subtype and size. These factors are also included in prediction tools such as the Sarculator and PERSARC. (18, 19) The respondents of this study were predominantly positive about using such prediction tools to select patients for perioperative treatment. Interestingly, genetic prognostic markers are less widely used in the identification of high-risk patients, whereas genetic prognostic markers seem promising in the identification of high-risk patients. Chibon et al. (20) showed that the gene expression profile CINSARC was a strong independent predictor for progressive disease and might identify high-risk patients that benefit from CTX. (21, 22)

Physicians seem to use different factors as indicators for RTX compared to CTX, which makes sense considering RTX aims to improve local control, whereas CTX aims to prevent distant disease. Surgical margins play an important role in the indication for RTX, as shown by Wasif et al.. (23) In contrast, the most important factor in the indication for CTX is histologic subtype. Physicians would consider perioperative CTX the most frequently for synovial sarcoma and rhabdomyosarcoma. The importance of using these factors in the indication for RTX and CTX provides an interesting insight in the clinical decision-making process of physicians. This could

be helpful for future studies because it quantifies the importance of adjusting for these factors in any observational study analysing the role of perioperative treatment.

The variation in administration of perioperative treatment among specialties and continents might arise from the lack of available evidence on eSTS management that may be sufficient to standardize clinical decision-making. The rarity of eSTS makes it challenging to conduct well-powered trials of perioperative treatment. Also, the multiple biologic subtypes, anatomic variability, and limited understanding of tumour biology and the tumour immune microenvironment of multiple subtypes impose difficulties on clinical trial design compared with clinical trials of perioperative treatment for other more prevalent cancers with more homogeneous populations. However, the variation in perioperative treatment also might arise from less knowledge of the literature outside a practitioner's clinical domain. (23, 24) In addition, the clinical guidelines leave room for interpretation and variation. (4, 5) These factors reflect the importance of a multidisciplinary expert board by reaching consensus decisions and to facilitate personalized sarcoma care.

Only a few studies have investigated the optimal routine follow-up policy of patients with localized high-grade eSTS. (25, 26) Therefore, the optimal frequency and intensity of the routine follow-up policy remains unclear. The current clinical guidelines recommend follow-up every 3-4 months in the first 2-3 years, then twice a year up to the fifth year and once a year thereafter. (4, 5) The guidelines do not specify whether chest CT scan or chest X ray should be used during follow-up. This study showed that physicians in North America have a clear preference for chest CT scan over chest X ray whereas in Asia and Europe no preference between these modalities was found. The variability of follow-up strategies found in this study and in other studies demonstrates the urgent need for well-designed prospective studies on follow-up evaluation. (27-30)

This study had some limitations. Only closed-ended questions were used to minimize the completion time and to maximize the completion rate. This resulted in a simplification of the responses. To prevent a lack of depth in the questionnaire and to prevent question order bias, a broad range of answers were included and arranged alphabetically. We recognize that other variables not captured in the questionnaire may also influence the choice for perioperative treatment.

Additionally, the use of a survey has the inherent limitation of selection bias because only physicians inclined to respond took time to do so. Also, the survey was sent to only active members of selected sarcoma societies, with some continents and specialties underrepresented in this study, which might affect the generalizability of our results.

Although electronic dissemination of the survey enables easy delivery and reply, many e-mail addresses were invalid and many e-mails were bounced back from e-mail filters. This might partially explain our moderate response rate of 31%. The high response rate (79%) of those who did open the e-mail shows that once the e-mail received the respondents, most went on and filled out the survey.

Conclusion

Although several studies have shown that adherence to clinical guidelines results in better patient outcomes, this study shows remarkable variation in the management of eSTS. Specialty and continent are important factors contributing to the variation in clinical practice, treatment recommendations and surveillance of patients with primary resectable high-grade eSTS.

References

1. WHO Classification of Tumours: Soft Tissue and Bone Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020.
2. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. European journal of cancer (Oxford, England : 1990). 2013;49(3):684-95.
3. Mastrangelo G, Coindre JM, Ducimetière F, Dei Tos AP, Fadda E, Blay JY, et al. Incidence of soft tissue sarcoma and beyond: a population-based prospective study in 3 European regions. Cancer. 2012;118(21):5339-48.
4. Von Mehren M, Kane JM, Agulnik M, Bui MM, Ganjoo KN, George S, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. 2018;16(5):536-63.
5. Casali PG, Abecassis N, Aro HT, Bauer S, Biagini R, Bielack S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2018;29(Suppl 4):iv51-iv67.
6. Ray-Coquard I, Thiesse P, Ranchère-Vince D, Chauvin F, Bobin JY, Sunyach MP, et al. Conformity to clinical practice guidelines, multidisciplinary management and outcome of treatment for soft tissue sarcomas. Annals of oncology : official journal of the European Society for Medical Oncology. 2004;15(2):307-15.
7. Derbel O, Heudel PE, Cropet C, Meeus P, Vaz G, Biron P, et al. Survival impact of centralization and clinical guidelines for soft tissue sarcoma (A prospective and exhaustive population-based cohort). PLoS One. 2017;12(2):e0158406.
8. Heudel PE, Cousin P, Lurkin A, Cropet C, Ducimetiere F, Collard O, et al. Territorial inequalities in management and conformity to clinical guidelines for sarcoma patients: an exhaustive population-based cohort analysis in the Rhône-Alpes region. International journal of clinical oncology. 2014;19(4):744-52.
9. Bagaria SP, Chang YH, Gray RJ, Ashman JB, Attia S, Wasif N. Improving Long-Term Outcomes for Patients with Extra-Abdominal Soft Tissue Sarcoma Regionalization to High-Volume Centers, Improved Compliance with Guidelines or Both? Sarcoma. 2018;2018:8141056.
10. Rossi CR, Vecchiato A, Mastrangelo G, Montesco MC, Russano F, Mocellin S, et al. Adherence to treatment guidelines for primary sarcomas affects patient survival: a side study of the European CONnective TIssue CAncer NETwork (CONTICANET). Annals of oncology : official journal of the European Society for Medical Oncology. 2013;24(6):1685-91.
11. Pasquali S, Bonvalot S, Tzanis D, Casali PG, Trama A, Gronchi A. Treatment challenges in and outside a network setting: Soft tissue sarcomas. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2019;45(1):31-9.
12. Acem I, Verhoef C, Rueten-Budde AJ, Grünhagen DJ, van Houdt WJ, van de Sande MAJ. Age-related differences of oncological outcomes in primary extremity soft tissue sarcoma: a multistate model including 6260 patients. European journal of cancer (Oxford, England : 1990). 2020;141:128-36.
13. R Development Core Team. R: A language and environment for statistical computing. 3.6.3 ed. Vienna, Austria: R Foundation for Statistical Computing; 2010.
14. Ngan R, Wang E, Porter D, Desai J, Prayogo N, Devi B, et al. Soft-tissue sarcomas in the Asia-Pacific region: a systematic review. Asian Pacific journal of cancer prevention : APJCP. 2013;14(11):6821-32.

15. Atun R, Jaffray DA, Barton MB, Bray F, Baumann M, Vikram B, et al. Expanding global access to radiotherapy. The Lancet Oncology. 2015;16(10):1153-86.
16. Pasquali S, Pizzamiglio S, Touati N, Litiere S, Marreaud S, Kasper B, et al. The impact of chemotherapy on survival of patients with extremity and trunk wall soft tissue sarcoma: revisiting the results of the EORTC-STBSG 62931 randomised trial. European journal of cancer (Oxford, England : 1990). 2019;109:51-60.
17. Zaidi MY, Ethun CG, Tran TB, Poultsides G, Grignol VP, Howard JH, et al. Assessing the Role of Neoadjuvant Chemotherapy in Primary High-Risk Truncal/Extremity Soft Tissue Sarcomas: An Analysis of the Multi-institutional U.S. Sarcoma Collaborative. Annals of surgical oncology. 2019;26(11):3542-9.
18. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Development and external validation of two nomograms to predict overall survival and occurrence of distant metastases in adults after surgical resection of localised soft-tissue sarcomas of the extremities: a retrospective analysis. The Lancet Oncology. 2016;17(5):671-80.
19. van Praag VM, Rueten-Budde AJ, Jeys LM, Laitinen MK, Pollock R, Aston W, et al. A prediction model for treatment decisions in high-grade extremity soft-tissue sarcomas: Personalised sarcoma care (PERSARC). European journal of cancer (Oxford, England : 1990). 2017;83:313-23.
20. Chibon F, Lagarde P, Salas S, Pérot G, Brouste V, Tirode F, et al. Validated prediction of clinical outcome in sarcomas and multiple types of cancer on the basis of a gene expression signature related to genome complexity. Nature medicine. 2010;16(7):781-7.
21. Valentin T, Lesluyes T, Le Guellec S, Chibon F. Chemotherapy in localized soft tissue sarcoma: will we soon have to treat grade 1 tumors? Update on CINSARC performances. Annals of oncology : official journal of the European Society for Medical Oncology. 2019;30(1):153-5.
22. Bertucci F, Finetti P, Monneur A, Birnbaum D. Pathological grade-independent prediction of chemosensitivity by CINSARC should rehabilitate adjuvant chemotherapy in soft tissue sarcomas of any grade. Annals of oncology : official journal of the European Society for Medical Oncology. 2019;30(2):342-3.
23. Wasif N, Smith CA, Tamurian RM, Christensen SD, Monjazeb AM, Martinez SR, et al. Influence of physician specialty on treatment recommendations in the multidisciplinary management of soft tissue sarcoma of the extremities. JAMA surgery. 2013;148(7):632-9.
24. Sherman KL, Wayne JD, Bilimoria KY. Overcoming specialty bias: another important reason for multidisciplinary management of soft tissue sarcoma. JAMA surgery. 2013;148(7):640.
25. Rothermundt C, Whelan JS, Dileo P, Strauss SJ, Coleman J, Briggs TW, et al. What is the role of routine follow-up for localised limb soft tissue sarcomas? A retrospective analysis of 174 patients. Br J Cancer. 2014;110(10):2420-6.
26. Hovgaard TB, Nymark T, Skov O, Petersen MM. Follow-up after initial surgical treatment of soft tissue sarcomas in the extremities and trunk wall. Acta oncologica (Stockholm, Sweden). 2017;56(7):1004-12.
27. Sakata K, Beitler AL, Gibbs JF, Kraybill WG, Virgo KS, Johnson FE. How surgeon age affects surveillance strategies for extremity soft tissue sarcoma patients after potentially curative treatment. The Journal of surgical research. 2002;108(2):227-34.
28. Johnson FE, Sakata K, Kraybill WG, Gibbs JF, Beitler AL, Sarkar S, et al. Long-term management of patients after potentially curative treatment of extremity soft tissue sarcoma: practice patterns of members of the Society of Surgical Oncology. Surgical oncology. 2005;14(1):33-40.
29. Beitler AL, Virgo KS, Johnson FE, Gibbs JF, Kraybill WG. Current follow-up strategies after potentially curative resection of extremity sarcomas: results of a survey of the members of the society of surgical oncology. Cancer. 2000;88(4):777-85.

30. Gerrand CH, Billingham LJ, Woll PJ, Grimer RJ. Follow up after Primary Treatment of Soft Tissue Sarcoma: A Survey of Current Practice in the United Kingdom. Sarcoma. 2007;2007:34128.

APPENDIX

Appendix A. Survey

What is the questionnaire about?
To investigate the variation in treatment policies regarding localized high grade (grade 2-3) soft tissue sarcomas of extremities (eSTS) and to get a better understanding of important patient and disease characteristics influencing disease management we would like to invite you to fill in this questionnaire.

The questionnaire consists of 20 questions. You can save the questionnaire at any time and complete it later. The questionnaire takes about 10 minutes to complete.

The questions in this questionnaire concern only primary (non-metastasized) high grade soft tissue sarcomas of extremities in adults (≥18 years). Additional treatment with isolated limb perfusion (ILP), immunotherapy and regional hyperthermia (RH) are not considered in this questionnaire.

Respondent characteristics
1. Are you a physician with an interest in soft tissue sarcomas? Yes/no

2. What is your speciality?
   a. Medical oncology
   b. Orthopaedic oncology
   c. Radiation oncology
   d. Surgical oncology
   e. Other

3. How many years have elapsed since completion of your fellowship?
   a. I am a fellow in-training
   b. < 5 years
   c. 5-10 years
   d. 11-15 years
   e. >15 years
   f. Other

4. Where do you practice?
   a. Africa
   b. Asia
   c. Australia/New Zealand/Oceania

d. Central/South America
e. Europe
f. North America

5. How many new cases of extremity soft tissue sarcoma do you treat in your hospital annually? (average experience over the last 5 years):
   a. < 5 per year
   b. 5-25 per year
   c. 25-50 per year
   d. > 50 per year

STS management

General

6. Which patient and/or disease characteristics do you use to distinguish between high-risk and low-risk STS patients on a scale of 1-5 (1: never, 5: always)?
   a. Age
   b. Depth
   c. Extent of tumour necrosis on MRI
   d. Gender
   e. Grade
   f. Histological subtype
   g. Infiltrative growth pattern
   h. Localization
   i. Mitotic rate
   j. Performance score (WHO/KPS)
   k. Presence of genetic prognostic marker(s)
   l. Presence of (other) oncological diseases in history
   m. Resectability: anticipated margin R0-R1-R2
   n. Size
   o. Tumour differentiation

Radiotherapy

7. What percentage of your patients with high-grade (grade 2-3) primary eSTS receive (neo)adjuvant radiotherapy? (scroll bar)

8. Which of the following patient and/or disease characteristics do generally influence your choice for treatment with (neo)adjuvant radiotherapy on a scale of 1-5 (1: never, 5: always)?
   a. Age
   b. Depth
   c. Extent of tumour necrosis on MRI
   d. Gender
   e. Grade
   f. Histological subtype
   g. Infiltrative growth pattern
   h. Localization
   i. Margin achieved: R0-R1-R2
   j. Mitotic rate
   k. Performance score (WHO/KPS)
   l. Presence of genetic prognostic marker(s)
   m. Presence of (other) oncological diseases in history
   n. Resectability: anticipated margin R0-R1-R2
   o. Size
   p. Tumour differentiation

9. When making decisions regarding radiotherapy in addition to surgery in patients with primary eSTS, at what cut-off value of the predicted 5-year local recurrence rate would you recommend (neo)adjuvant radiotherapy? (scroll bar)*

10. When making decisions regarding radiotherapy in addition to surgery in patients with primary eSTS, at what cut-off value of the absolute 5-year local recurrence rate reduction (ARR) would you recommend (neo)adjuvant radiotherapy? (scroll bar)*

11. Would you consider using a prediction tool for local recurrence, such as Sarculator or Persarc, to indicate (neo)adjuvant radiotherapy in eSTS patients? Yes/No

Chemotherapy

12. What percentage of your patients with high-grade (grade 2-3) primary eSTS receive (neo)adjuvant chemotherapy? (scroll bar)

13. Which of the following patient and/or disease characteristics do generally influence your choice for treatment with (neo)adjuvant chemotherapy on a scale of 1-5 (1= never, 5= always)?
   a. Age

b. Depth
c. Extent of tumour necrosis on MRI
d. Gender
e. Grade
f. Histological subtype
g. Infiltrative growth pattern
h. Localization
i. Margin achieved: R0-R1-R2
j. Mitotic rate
k. Performance score (WHO/KPS)
l. Presence of genetic prognostic marker(s)
m. Presence of (other) oncological diseases in history
n. Resectability: anticipated margin R0-R1-R2
o. Size
p. Tumour differentiation

14. For what predicted 5-year mortality risk do you consider (neo)adjuvant chemotherapy in addition to surgery in primary eSTS? (scroll bar)*

15. For what absolute 5-year mortality risk reduction (ARR) do you consider chemotherapy in addition to surgery in primary eSTS? (scroll bar)*

16. Do you feel there is sufficient evidence to use (neo)adjuvant chemotherapy for treatment of primary high-grade (grade 2-3) resectable eSTS? Yes/No

17. Would you consider using a prediction tool for overall survival and/or distant metastasis risk, such as Sarculator or Persarc, to indicate (neo)adjuvant chemotherapy in eSTS patients? Yes/No

18. In which STS histologic subtypes (grade 2/3, deep-seated, >5cm) would you generally consider (neo)adjuvant chemotherapy on a scale of 1-5 (1= never, 5= always)?
    a. Alveolar soft part sarcoma
    b. Angiosarcoma
    c. Dedifferentiated liposarcoma
    d. Epithelioid sarcoma
    e. Fibrosarcoma
    f. Leiomyosarcoma
    g. Malignant peripheral nerve sheath tumour (MPNST)
    h. Myxofibrosarcoma

i. Myxoid liposarcoma
j. Pleomorphic liposarcoma
k. Rhabdomyosarcoma
l. Round cell liposarcoma
m. Spindle cell sarcoma
n. Synovial sarcoma
o. Undifferentiated sarcoma

19. Would older age (>70 years) be an absolute contra-indication for (neo)adjuvant chemotherapy? Yes/No

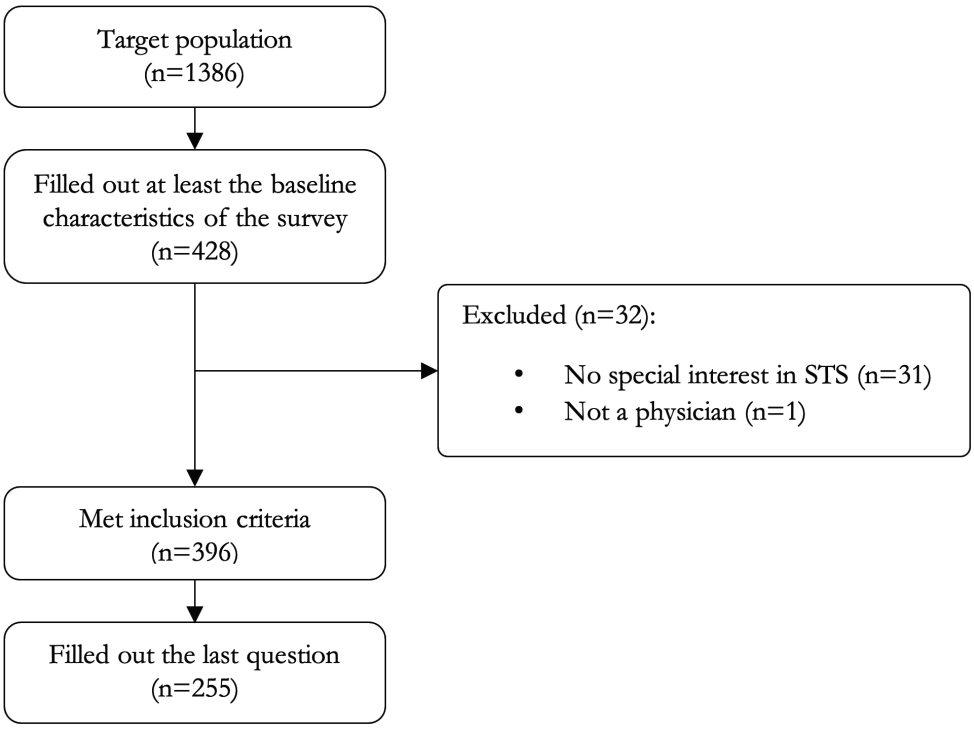
Follow-up

20. What is your follow-up schedule after initial treatment (with surgery and (neo) adjuvant treatment if indicated) has been completed for primary high-grade eSTS? Please enter the number of times during each time interval.

	1st year	2nd year	3rd year	4th year	5th year
Outpatient clinic visit					
X-thorax					
CT thorax					
X-extremity					
CT extremity					
MRI extremity					
PET-CT scan					

21. After how many years of disease free survival would you feel comfortable to end the follow-up of your patient with primary high-grade eSTS?

*The results of question 9, 10, 14, 15 have not been reported, as these questions were interpreted in multiple ways. Therefore, the results were not reliable.



Appendix B. Flowchart of the respondents

Appendix C. The use of patient and disease characteristics to distinguish between high-risk and low-risk eSTS patients stratified by specialty

	Mean 5-point Likert Scale Score (Standard error of the mean)					
	Overall	**Medical oncology**	**Orthopaedic oncology**	**Radiation oncology**	**Surgical oncology**	**p***
Age	2.66 (0.070)	2.61 (0.147)	2.69 (0.098)	2.08 (0.223)	2.83 (0.168)	0.090
Depth	4.09 (0.059)	4.31 (0.106)	4.18 (0.080)	3.64 (0.336)	3.84 (0.136)	**0.007**
Extent of tumour necrosis on MRI	3.25 (0.068)	2.88 (0.143)	3.53 (0.096)	2.71 (0.244)	3.45 (0.147)	**0.000**
Gender	1.52 (0.044)	1.37 (0.077)	1.54 (0.070)	1.33 (0.130)	1.72 (0.105)	**0.044**
Grade	4.93 (0.017)	4.93 (0.036)	4.91 (0.029)	5.00 (0.000)	4.92 (0.035)	0.602
Histological subtype	4.65 (0.033)	4.73 (0.056)	4.59 (0.051)	4.38 (0.189)	4.70 (0.068)	0.052
Infiltrative growth pattern	3.95 (0.058)	3.50 (0.117)	4.15 (0.079)	3.79 (0.225)	4.07 (0.136)	**0.000**
Localization	3.73 (0.059)	3.77 (0.108)	3.78 (0.086)	3.64 (0.233)	3.50 (0.160)	0.370
Mitotic rate	4.06 (0.054)	4.11 (0.103)	4.07 (0.079)	3.54 (0.233)	4.20 (0.121)	**0.046**
Performance score	2.86 (0.070)	3.00 (0.157)	2.84 (0.098)	2.83 (0.274)	2.88 (0.162)	0.833
Presence of genetic prognostic maker(s)	3.06 (0.066)	3.02 (0.148)	3.13 (0.094)	2.75 (0.250)	2.95 (0.151)	0.478
Presence of (other) oncological diseases in history	2.84 (0.067)	2.65 (0.142)	2.97 (0.095)	2.79 (0.282)	2.67 (0.153)	0.184
Resectability	4.40 (0.051)	4.43 (0.101)	4.45 (0.074)	4.46 (0.217)	4.20 (0.123)	0.337
Size	4.51 (0.043)	4.81 (0.059)	4.52 (0.056)	4.56 (0.164)	4.10 (0.132)	**0.000**
Tumour differentiation	4.40 (0.045)	4.20 (0.118)	4.46 (0.059)	4.40 (0.173)	4.51 (0.076)	0.088

*Global P value for difference in distribution among specialty

Appendix D. Perioperative therapy in a cohort of high-grade eSTS patients

	Overall (N=6260)	**Asia (N=1850)**	**Europe (N=3304)**	**North America[#] (N=1106)**	**P***
Surgical margin					<0.001
R0	5338 (87.9%)	1764 (95.4%)	2630 (84.5%)	944 (85.4%)	
R1-R2	732 (12.1%)	86 (4.6%)	484 (15.5%)	162 (14.6%)	
Missing	*190*	*0*	*190*	*0*	
Radiotherapy					<0.001
0	3016 (48.2%)	1488 (80.4%)	1247 (37.8%)	281 (25.5%)	
1	3239 (51.8%)	362 (19.6%)	2055 (62.2%)	822 (74.5%)	
Missing	*5*	*0*	*2*	*3*	
Chemotherapy					<0.001
0	5240 (83.7%)	1283 (69.4%)	2889 (87.4%)	1068 (96.7%)	
1	1019 (16.3%)	567 (30.6%)	415 (12.6%)	37 (3.3%)	
Missing	*1*	*0*	*0*	*1*	

*Global P value for difference in distribution among continents

[#]Only data of one centre in North America was available

Appendix E. Patient and disease characteristics influencing RTX recommendation stratified by specialty

	Mean 5-point Likert Scale Score (Standard error of the mean)					
	Overall	**Medical oncology**	**Orthopaedic oncology**	**Radiation oncology**	**Surgical oncology**	**P***
Age	2.89 (0.076)	2.79 (0.158)	2.91 (0.117)	2.95 (0.270)	2.82 (0.139)	0.912
Depth	3.96 (0.071)	4.20 (0.139)	3.94 (0.105)	3.95 (0.301)	3.78 (0.157)	0.270
Extent of tumour necrosis on MRI	2.72 (0.074)	2.70 (0.164)	2.65 (0.111)	2.74 (0.274)	2.90 (0.152)	0.710
Gender	1.38 (0.044)	1.26 (0.073)	1.38 (0.065)	1.21 (0.123)	1.58 (0.131)	0.097
Grade	4.59 (0.047)	4.77 (0.083)	4.46 (0.078)	4.65 (0.209)	4.69 (0.066)	**0.046**
Histological subtype	4.44 (0.049)	4.47 (0.103)	4.36 (0.076)	4.30 (0.206)	4.65 (0.078)	0.178
Infiltrative growth pattern	3.94 (0.070)	3.39 (0.167)	4.20 (0.090)	3.95 (0.270)	3.98 (0.155)	**0.000**
Localization	4.16 (0.060)	4.21 (0.123)	4.17 (0.087)	4.20 (0.156)	4.02 (0.160)	0.774
Margin achieved	4.58 (0.055)	4.62 (0.113)	4.52 (0.090)	4.60 (0.210)	4.67 (0.078)	0.785
Mitotic rate	3.41 (0.076)	3.57 (0.164)	3.22 (0.112)	2.95 (0.259)	3.82 (0.153)	**0.008**
Performance score	3.00 (0.074)	3.33 (0.157)	2.70 (0.105)	3.32 (0.230)	3.34 (0.166)	**0.001**
Presence of genetic prognostic maker(s)	2.44 (0.071)	2.46 (0.154)	2.35 (0.098)	2.32 (0.254)	2.68 (0.182)	0.382
Presence of (other) oncological diseases in history	2.53 (0.073)	2.82 (0.173)	2.28 (0.100)	2.84 (0.245)	2.54 (0.146)	**0.013**
Resectability	4.63 (0.042)	4.71 (0.093)	4.61 (0.060)	4.45 (0.223)	4.65 (0.073)	0.526
Size	4.34 (0.056)	4.60 (0.107)	4.26 (0.085)	4.35 (0.221)	4.20 (0.122)	0.063
Tumour differentiation	3.99 (0.067)	3.97 (0.156)	3.88 (0.099)	3.70 (0.282)	4.37 (0.093)	**0.039**

*Global P value for difference in distribution among specialties

Appendix F. Patient and disease characteristics influencing CTX recommendation stratified by specialty

	Mean 5-point Likert Scale Score (Standard error of the mean)					
	Overall	**Medical oncology**	**Orthopaedic oncology**	**Radiation oncology**	**Surgical oncology**	**P***
Age	3.94 (0.073)	4.07 (0.136)	4.02 (0.106)	4.26 (0.263)	3.54 (0.171)	0.055
Depth	3.58 (0.082)	3.97 (0.158)	3.60 (0.116)	3.72 (0.351)	2.83 (0.188)	**0.000**
Extent of tumour necrosis on MRI	2.81 (0.081)	2.67 (0.165)	3.02 (0.115)	2.39 (0.315)	2.71 (0.188)	0.112
Gender	1.40 (0.046)	1.38 (0.098)	1.43 (0.066)	1.22 (0.129)	1.39 (0.120)	0.752
Grade	4.55 (0.057)	4.69 (0.114)	4.50 (0.081)	4.63 (0.232)	4.41 (0.153)	0.394
Histological subtype	4.73 (0.038)	4.76 (0.087)	4.70 (0.050)	4.83 (0.090)	4.69 (0.110)	0.803
Infiltrative growth pattern	3.11 (0.084)	2.96 (0.171)	3.29 (0.122)	2.57 (0.305)	2.98 (0.208)	0.101
Localization	3.36 (0.084)	3.73 (0.156)	3.32 (0.122)	3.22 (0.348)	2.85 (0.202)	**0.012**
Margin achieved	3.56 (0.084)	3.65 (0.162)	3.53 (0.126)	3.72 (0.266)	3.39 (0.203)	0.745
Mitotic rate	3.57 (0.082)	3.81 (0.158)	3.55 (0.124)	2.83 (0.259)	3.56 (0.198)	0.053
Performance score	3.87 (0.079)	4.37 (0.122)	3.58 (0.118)	4.32 (0.276)	3.85 (0.210)	**0.000**
Presence of genetic prognostic maker(s)	2.94 (0.084)	2.79 (0.169)	3.06 (0.123)	2.78 (0.275)	2.93 (0.200)	0.559
Presence of (other) oncological diseases in history	2.95 (0.081)	3.11 (0.171)	2.87 (0.115)	3.06 (0.221)	2.78 (0.199)	0.534
Resectability	3.77 (0.078)	4.00 (0.154)	3.68 (0.115)	3.94 (0.249)	3.51 (0.204)	0.193
Size	4.20 (0.068)	4.57 (0.114)	4.10 (0.098)	4.58 (0.221)	3.62 (0.190)	**0.000**
Tumour differentiation	3.97 (0.074)	3.89 (0.170)	4.01 (0.103)	3.74 (0.295)	4.09 (0.155)	0.671

*Global P value for difference in distribution among specialties

Chapter 9

The Role of Perioperative Chemotherapy in Primary High-Grade Extremity Soft Tissue Sarcoma: A Risk-Stratified Analysis using PERSARC

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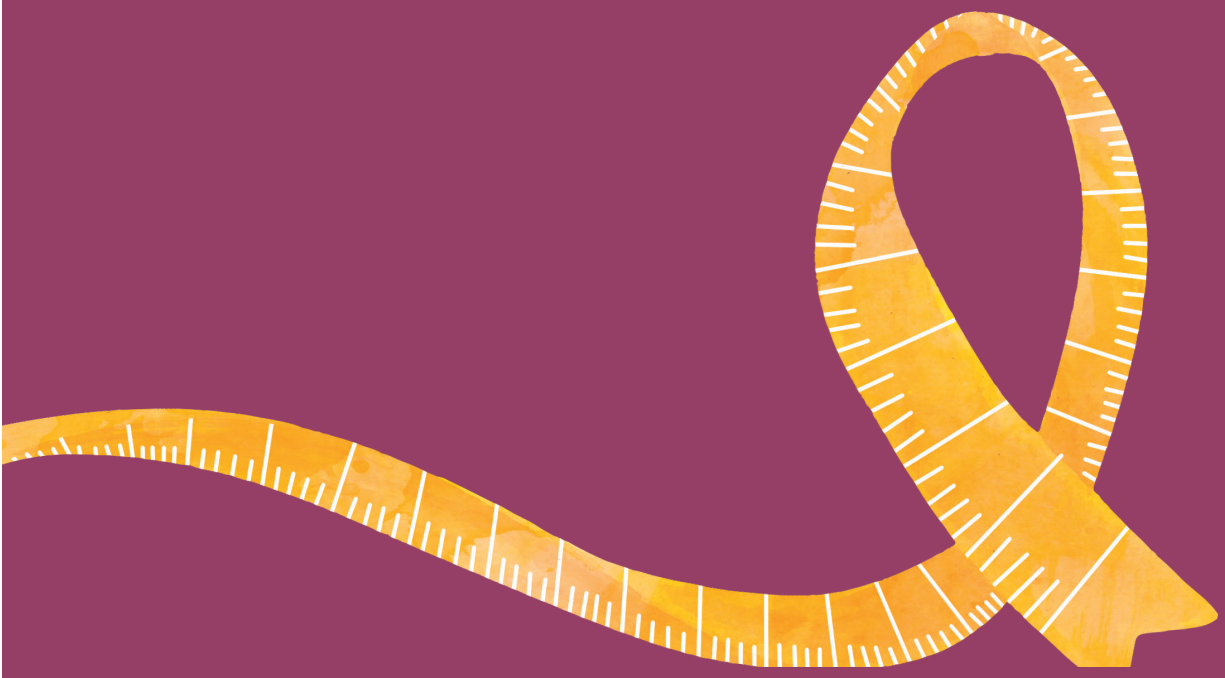

Abstract

Background

The aim of the study is to assess the effect of perioperative chemotherapy (CTX) in patients with grade II-III extremity soft tissue sarcoma (eSTS) on overall survival (OS) and evaluate whether the PERSARC prediction tool could identify patients with eSTS more likely to benefit from CTX.

Methods

Patients (18-70 years) with primary high-grade eSTS surgically treated with curative intent were included in the retrospective cohort study. The effect of any perioperative CTX and anthracycline+ifosfamide (AI)-based CTX on OS was investigated in three PERSARC-risk groups (high/intermediate/low). The PERSARC-risk groups were defined by the 33% and 66% quantile of the predicted 5-year OS of the study population equal to a 5-year OS of 65.8% and 79.8%, respectively. The effect of CTX on OS was investigated with weighted Kaplan-Meier curves and multivariable Cox models with an interaction between risk group and CTX.

Results

This study included 5683 patients. The weighted Kaplan-Meier curves did not demonstrate a beneficial effect of any CTX and AI-based CTX on OS in the overall population. However, in the high PERSARC-risk group the 5-year OS of AI-based CTX was significantly better than no CTX (69.8% vs 59.0%, respectively, p=0.004) (HR 0.66, 95%CI 0.53–0.83).

Conclusion

This study demonstrated a beneficial effect of AI-based CTX on OS in a selected group of high-risk patients with an absolute survival benefit of 11% as stratified by the PERSARC prediction tool. However, no beneficial effect of CTX on OS was found in the overall population of patients with primary high-grade eSTS younger than 70 years.

Introduction

Soft tissue sarcomas (STS) are rare tumours of mesenchymal origin with various histologic and clinical features, with an estimated incidence of 4.7 per 100.000 persons in Northern Europe. (1) The mainstay of treatment for patients with primary extremity STS (eSTS) is surgery, often accompanied by radiotherapy (RTX). (2) However, approximately 30% of the patients with eSTS will eventually develop distant metastasis (DM) within 5 years. (3) Therefore, perioperative chemotherapy (CTX) is increasingly considered in patients with high-risk eSTS worldwide, in order to prevent future metastatic disease and improve survival rates.

The rarity and heterogeneity of eSTS, however, poses significant difficulties in demonstrating the beneficial effect of perioperative CTX in eSTS patients, and especially to identify patients that are more likely to benefit from CTX. Despite the efforts of several studies, the level of evidence for perioperative CTX remains debated. Post-hoc analyses within recent trials showed a beneficial effect of adjuvant CTX with anthracycline and ifosfamide (AI) in patients with a low predicted overall survival (OS) suggesting that beneficial outcomes of perioperative CTX are particularly limited to a selected group of high-risk patients. (4-7) High-risk patients are defined as patients with a higher grade and worse predicted survival. Consequently, greater improvement in survival and thus potentially a higher efficacy of CTX is to be expected in the high-risk group, when compared to patients with a more favourable risk profile. (4, 8) Therefore, we hypothesised that the effect of perioperative CTX differs within different subgroups of baseline risk.

The primary aim of this study was to assess the effect of all CTX regimens in general and of AI-based CTX specifically on OS compared to local treatment alone, in a large cohort of patients with primary high-grade eSTS (FNCLCC grade II and III), who were surgically treated with curative intent. The secondary aim was to identify whether the potential benefit of perioperative CTX varies between low-, intermediate-, and high-risk patients, as defined by the PERSARC prediction tool, an externally validated prediction tool for primary high-grade eSTS. (9-11)

Methods

Study design

The effect of CTX was investigated in a retrospective cohort of patients with high-grade eSTS, in accordance with the PATH-statement. (12) The PATH-statement outlines a set of principles and criteria for predictive approaches to heterogeneity

of treatment effect (HTE) analyses. The study cohort contained data from multiple specialized sarcoma centres (**Appendix A**).

Data were collected between January 1st, 2000, and 31st December, 2016, except for the data from the EORTC trial 62931 which were collected between February 1995 and December 2003. (13) Ethical approval was obtained by the institutional review board prior to the study (G20.006).

The primary outcome was OS defined as the time from surgery until death of any cause or last recorded follow-up.

Participants

Adults (18-70 years) with primary high-grade (FNCLCC grade II and III (14)) eSTS surgically treated with curative intent with correctly registered time-to-events were included in this study. Patients were excluded if they had a Kaposi's sarcoma or alveolar rhabdomyosarcoma or received isolated limb perfusion as perioperative treatment. Patients with spindle cell sarcoma were excluded in our analyses as they were underrepresented in the perioperative AI-based CTX-group (1 out of 92 patients). In addition, patients older than 70 years were excluded in this analysis, as older age is often a contra-indication for perioperative chemotherapy. (15)

Variables

The primary variable of interest was CTX (yes/no). Neoadjuvant and adjuvant CTX were grouped together as one category. All CTX regimens were included, independent of specific drugs, the number of cycles or dose. The other variables considered in this analysis were: age at definitive surgery (years), size (cm), depth (deep/superficial), grade, (II/III), surgical margins (R0/R1-R2), RTX (neoadjuvant/adjuvant/no RTX), and histological subtype. A detailed description of the definitions of each variable could be found in **Appendix A**.

Another variable considered was the 5-year predicted OS which was predicted using the PERSARC prediction tool. (9) The 33% and 66% quantiles of the predicted probabilities were used to create three PERSARC-risk groups; 5-year predicted OS <33% quantile was high PERSARC-risk, 33-66% quantile was intermediate PERSARC-risk, and ≥66% quantile was low PERSARC-risk. PERSARC includes the variables: age, size, depth, grade, histology, surgical margin, and RTX.

Statistical analysis

Baseline characteristics were described with proportions for categorical variables and means with standard deviations or medians with interquartile ranges for normally distributed and non-normally distributed continuous variables, respectively. Differences in categorical variables were tested with the Chi-square test or Fisher's exact test. Differences in continuous variables were tested with the Student's t-test.

The effect of any CTX and AI-based CTX was investigated in the overall population and in the three risk groups (high/intermediate/low), based on the PERSARC prediction tool.

Median follow-up was assessed with the reverse Kaplan-Meier method. (16) The effect of CTX in the PERSARC-risk groups was investigated with crude Kaplan-Meier curves (cKMs), weighted Kaplan-Meier curves (wKMs) and a multivariable Cox proportional hazards model.

wKMs for OS were used to compare patients who received CTX and those who did not. (17) Weights were computed using the Inverse Probability of Treatment Weighting (IPTW) approach. Within each PESARC-risk group the distribution of covariates was modelled with a logistic regression model with CTX (yes/no) as the outcome variable (**Appendix C Table 1, Appendix E Table 1**). The included variables in this model were: age, tumour size, depth, histology, grade, surgical margin and RTX. Based on this model weights were computed for each patient to create a weighted data set (**Appendix C Figure 1, Appendix E Figure 1**). Differences in OS were evaluated with the log-rank test.

Multivariable Cox proportional hazards models were used to estimate the effect of CTX on OS adjusted for age, tumour size, depth, histology, grade, surgical margin and RTX. An interaction term between CTX (yes/no) and PERSARC-risk group (high/intermediate/low) was included in the model to investigate the effect of CTX per risk group.

Multiple imputation for missing covariates was applied using the 'mice' package in R (version 4.0.3) with 20 imputations. The results were pooled using Rubin's rule. (18)

All analyses were performed in the R-software environment and a p-value of less than 0.05 was considered significant. (19)

Results

This study included 5683 patients with primary high-grade eSTS. The median follow-up was 5.21 years (95% CI 5.11-5.31). The mean age was 52 years. The PERSARC prediction tool was used to predict the 5-year OS probability from baseline for each patient. The predicted 5-year OS ranged between 11.8% and 96.4% with a median of 73.4%. The PERSARC-risk groups were defined by the 33% and 66% quantile of these predicted 5-year OS probabilities equal to a 5-year OS of 65.8% and 79.8%, respectively (**Appendix B Figure 1**). Twenty-nine percent of the overall population (n=1635) received perioperative CTX. In the high, intermediate, and low PERSARC-risk group 38.7% (n=735 out of 1897), 31.1% (n=590 out of 1897), and 16.4% (n=310 out of 1889) received perioperative CTX, respectively. **Table 1** provides an overview of the patient characteristics in the no CTX and CTX-group. **Appendix B Table 1** provides an overview of the patient characteristics in the overall population and per PERSARC-risk group. Patients who received perioperative CTX were younger, had larger and more grade III tumours (**Table 1, Appendix C Table 1**).

Table 1. Baseline characteristics in patients with and without (AI-based) CTX

	Overall (N=5683)	**No CTX (N=4047)**	**CTX (all regimens) (N=1635)**	**AI-based CTX (N=1036)**	**No vs all CTX P***	**No vs AI-based CTX P***
Baseline predicted 5-year OS (%)					<0.001	<0.001
Mean (SD)	70.7 (15.3)	72.4 (15.2)	66.3 (14.4)	67.5 (13.9)		
Median (IQR)	73.4 (61.2-82.9)	76.0 (63.7-84.1)	67.9 (57.0-77.4)	68.9 (58.7-78.2)		
Age at surgery (years)					<0.001	<0.001
Mean (SD)	51.7 (13.6)	52.9 (13.3)	48.6 (13.9)	47.9 (13.7)		
Median (IQR)	54 (42-63)	56 (44-64)	50 (38-61)	49 (38-60)		
Size (cm)					<0.001	<0.001
Mean (SD)	8.60 (5.60)	8.14 (5.63)	9.74 (5.36)	9.47 (5.41)		
Median (IQR)	7.0 (4.5-11.0)	6.7 (4.0-10.5)	9.0 (6.0-12.0)	8.0 (5.7-12.0)		
Missing	*298*	*229*	*69*	*48*		
Depth					<0.001	<0.001
Deep	3902 (72.6%)	2605 (67.7%)	1297 (84.9%)	899 (86.9%)		
Superficial	1476 (27.4%)	1245 (32.3%)	230 (15.1%)	135 (13.1%)		
Missing	*305*	*197*	*108*	*2*		
Histological subtype					<0.001	<0.001
LMS	589 (10.4%)	426 (10.5%)	163 (10.0%)	115 (11.1%)		
LPS	1180 (20.8%)	850 (21.0%)	330 (20.2%)	243 (23.5%)		
MFS	786 (13.8%)	646 (16.0%)	140 (8.6%)	52 (5.0%)		

	Overall (N=5683)	No CTX (N=4047)	CTX (all regimens) (N=1635)	AI-based CTX (N=1036)	No vs all CTX P*	No vs AI-based CTX P*
UPS and NOS	1515 (26.7%)	1073 (26.5%)	441 (27.0%)	265 (25.6%)		
MPNST	368 (6.5%)	275 (6.8%)	93 (5.7%)	49 (4.7%)		
SS	731 (12.9%)	393 (9.7%)	338 (20.7%)	237 (22.9%)		
Other	512 (9.0%)	383 (9.5%)	129 (7.9%)	75 (7.2%)		
Missing	*2*	*1*	*1*	*0*		
Grade						
2	847 (14.9%)	771 (19.1%)	75 (4.6%)	49 (4.7%)	<0.001[a]	<0.001[a]
3	2033 (35.8%)	1618 (40.0%)	415 (25.4%)	78 (7.5%)		
High grade not further specified	2803 (49.3%)	1658 (41.0%)	1145 (70.0%)	909 (87.7%)		
Margin						
R0	5066 (91.7%)	3545 (87.6%)	1520 (95.3%)	999 (97.2%)	<0.001	<0.001
R1-R2	457 (8.3%)	382 (9.4%)	75 (4.7%)	29 (2.8%)		
Missing	*160*	*120*	*40*	*8*		
Radiotherapy						
No radiotherapy	3026 (53.6%)	2024 (50.3%)	1002 (62.0%)	709 (68.4%)	<0.001	<0.001
adjuvant	1674 (29.7%)	1214 (30.1%)	460 (28.4%)	234 (22.6%)		
neoadjuvant	923 (16.4%)	772 (19.2%)	151 (9.3%)	91 (8.8%)		
Neo- and adjuvant	21 (0.4%)	17 (0.4%)	4 (0.2%)	2 (0.2%)		
Missing	*39*	*20*	*18*	*0*		

OS: overall survival, SD: standard deviation, IQR: interquartile range, LMS: leiomyosarcoma, LPS: liposarcoma, MFS: myxofibrosarcoma, UPS: undifferentiated pleomorphic sarcoma, NOS: (pleomorphic) soft tissue sarcomas not-otherwise-specified, MPNST: malignant peripheral nerve sheet tumour, SS: synovial sarcoma, CTX: chemotherapy

*Global P value for difference in distribution between CTX and no CTX-group. [a]Based on the multiply imputed dataset

All chemotherapy regimens

Overall survival

Figure 1 and **2** display the cKM and wKM stratified by CTX for the overall population and for the PERSARC-risk groups, respectively. There was no significant difference in the survival curve between patients who received CTX and who did not receive CTX in the overall population (p=0.663). However, a significant difference in OS in favour of CTX was found in the high PERSARC-risk group with a p-value of 0.018 (**Figure 2**).

The 5-year OS for the CTX-group was 75.8% versus 77.3% for the no CTX-group (p=0.405). In the low PERSARC-risk group the 5-year OS for the CTX-group and no CTX-group was 88.0% and 95.0% (p=0.055); in the intermediate PERSARC-risk

group, the 5-year OS was 81.9% and 78.2% (p=0.138), and in the high PERSARC-risk group, the 5-year OS was 61.0% and 58.4% (p=0.375), respectively (**Table 2**).

After adjustment for age, size, depth, histology, grade, margin, and RTX, no difference in CTX effect on OS in the low and intermediate PERSARC-risk groups could be found in the multivariable cox model (HR 1.27 (95% CI 0.878-1.84) and 0.803 (95% CI 0.631-1.02), respectively). The HR in the high-risk group was 0.822 (95% CI 0.697-0.969).

Anthracycline and ifosfamide-based chemotherapy regimen only

Of the 1635 patients who received perioperative CTX in this series, details about CTX regimen were available for 1259 patients of which 82.3% (n=1036) received AI-based CTX (**Table 1**). **Appendix D Table 1** provide an overview of the patient characteristics per PERSARC-risk group.

Overall survival in patients who received AI-based CTX

Figure 3 and **4** display the cKM and wKM stratified by AI-based CTX for the overall population and for the PERSARC-risk groups, respectively. Patients who received AI-based CTX seemed to have a better OS than patients who did not receive CTX, but this was not statistically significant (p = 0.060) (**Figure 3**). A significant difference in OS in favour of AI-based CTX in the high PERSARC-risk group was found with a p-value of <0.001. No difference in OS was found in the low and intermediate PERSARC-risk groups (p=0.422 and p=0.181, respectively) (**Figure 4**).

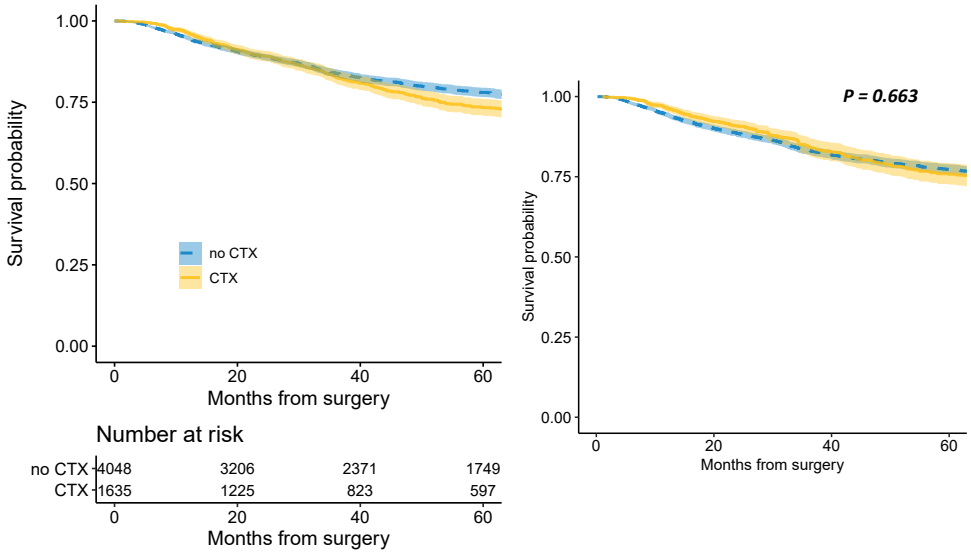

Figure 1. Crude (left) and weighted (right) Kaplan-Meier of OS stratified by CTX in the overall population

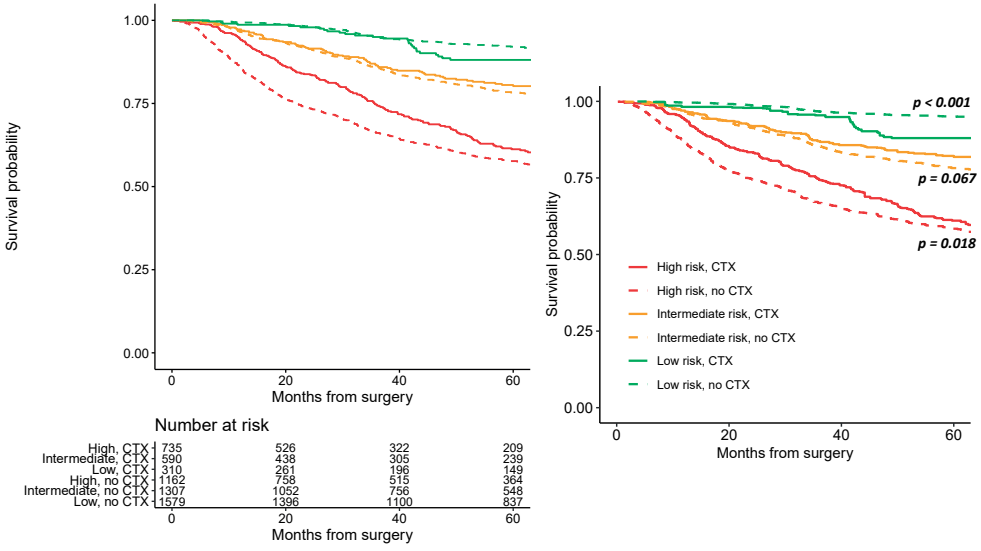

Figure 2. Crude (left) and weighted (right) Kaplan-Meier of OS for high, intermediate and, low PERSARC-risk patients stratified by CTX administration

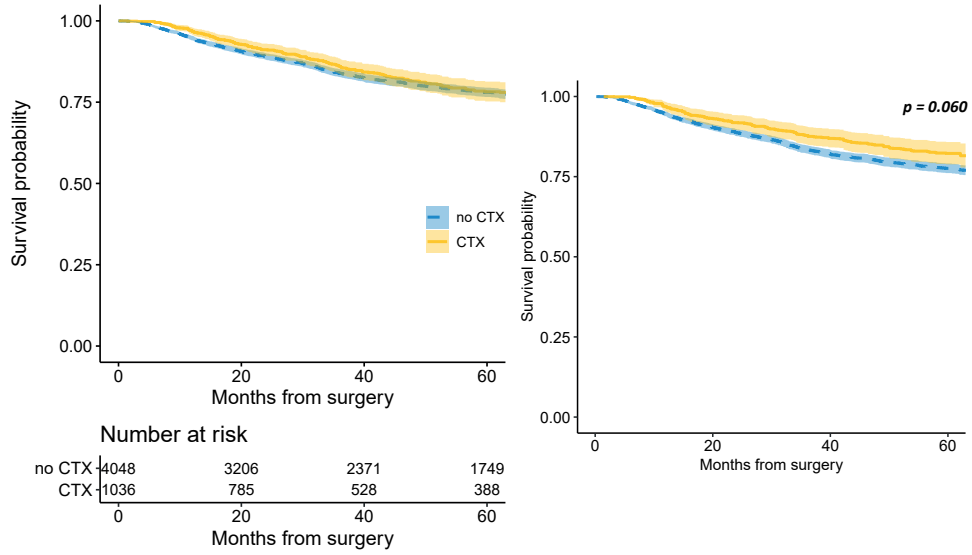

Figure 3. Crude (left) and weighted (right) Kaplan-Meier of OS stratified by AI-based CTX in the overall population

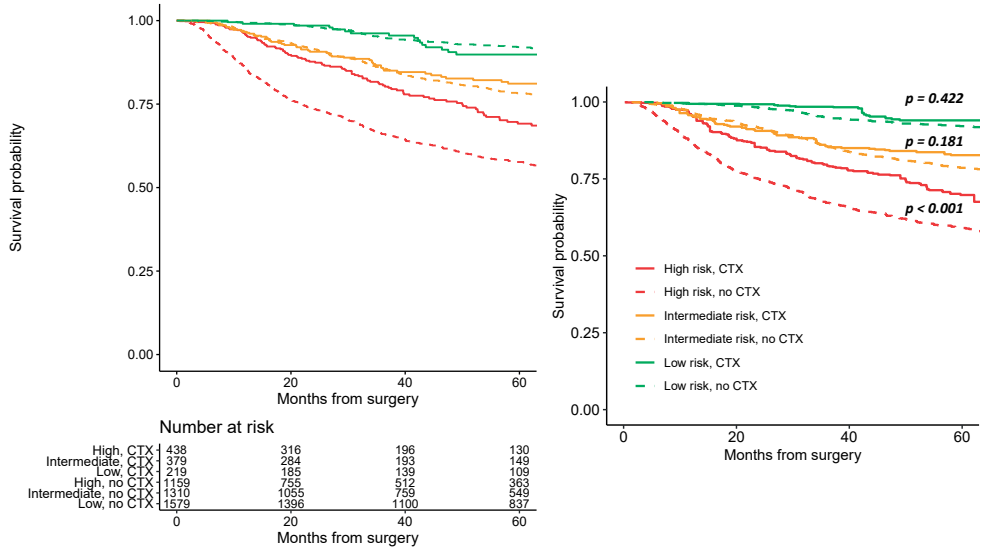

Figure 4. Crude (left) and weighted (right) Kaplan-Meier of OS stratified by AI-based CTX administration and PERSARC-risk group

The 5-year OS for the AI-based CTX-group was 82.2% versus 77.6% for the no CTX-group (p=0.014). In the high PERSARC-risk group the absolute risk difference in 5-year OS between the AI-based CTX-group and no CTX-group was 10.7 percentage

points in favour of AI-based CTX (p=0.004). There was no significant difference in 5-year OS in the low and intermediate PERSARC-risk group (**Table 2**).

Table 2. 5-year OS for the overall population stratified by PERSARC-risk group and CTX

	5-year OS (95% CI)		**Absolute risk difference (95% CI)**	
	All CTX	**No CTX#**		***P***
Overall population	75.8 (72.5 - 79.2)	77.3 (75.8 - 78.9)	-1.52 (-5.10 – 2.06)	0.405
High risk	61.0 (56.3 – 66.2)	58.4 (55.1 – 62.0)	2.63 (-3.18 – 8.44)	0.375
Intermediate risk	81.9 (77.8 – 86.1)	78.2 (75.5 – 81.0)	3.65 (-1.17 – 8.46)	0.138
Low risk	88.0 (82.0 – 94.4)	95.0 (91.1 – 99.2)	-7.03 (-14.2 – 0.144)	0.055
	AI-based CTX	**No CTX***		***P***
Overall population	82.2 (78.9 - 85.8)	77.6 (76.1 - 79.1)	4.65 (0.935 – 8.37)	0.014
High risk	69.8 (63.3 – 76.9)	59.0 (55.7 – 62.5)	10.7 (3.48 – 18.0)	0.004
Intermediate risk	82.7 (77.9 – 87.8)	78.6 (76.0 – 81.2)	4.12 (-1.33 – 9.58)	0.139
Low risk	94.0 (89.7 – 98.6)	92.1 (90.6 – 93.7)	1.88 (-2.70 – 6.46)	0.421

#Based on the wKM of all CTX regimens

*Based on the wKM of AI-based CTX regimens only

OS: overall survival, CTX: chemotherapy, CI: confidence interval

After adjustment for the baseline characteristics, the HR of AI-based CTX on OS was 0.661 (95% CI 0.527 – 0.828) in the high PERSARC-risk group, 0.813 (95% CI 0.608 – 1.09) in the intermediate PERSARC-risk group and, 1.00 (95% CI 0.616 –1.62) in the low PERSARC-risk group.

Discussion

The present study exploring the role of perioperative CTX in primary high-grade eSTS did not demonstrate a beneficial effect of any CTX on OS in the overall population. However, perioperative AI-based CTX led to improved OS for patients with a high PERSARC-risk profile, with a 5-year absolute risk reduction in mortality of 11%.

To our knowledge this is the largest multicentre cohort study to date examining the effect of perioperative CTX in patients with primary high-grade eSTS. The strength of this study is that we only included high-grade eSTS and used a validated multivariable risk-based model to assess heterogeneity of treatment effect instead of conventional 'one-variable-at-the-time'-subgroup analyses. This allows us to reduce the risk of false-positives due to multiple comparisons and to better inform individual treatment decisions, since it accounts for the fact that patients have multiple characteristics that vary simultaneously. (12)

Despite several published randomised and non-randomised studies, the role of perioperative CTX for eSTS is still subject of discussion. (4, 13, 20-29) In 2008 a meta-analysis of 18 doxorubicin-based trials showed an absolute risk reduction of 6% on OS in patients with STS (at what time point was not reported). (27) To date, five randomised trials compared the effect of perioperative AI-based CTX versus no CTX in patients with STS. (13, 20-23) The 5-year OS in these studies ranged between 65 and 72% for the CTX arm, and between 47 and 69% for the no CTX arm. Furthermore, other more recent trials including an AI-based CTX arm, showed 5-year survival rates between 61 and 76% for the CTX group. (5, 30-32) In our cohort, the 5-year OS was 82% in the AI-based CTX group and 78% in the no CTX group. The higher 5-year OS in our cohort might be explained by an on average smaller tumour size compared with the abovementioned studies, as most studies included selection criteria for size. (5, 30, 31) Furthermore, most trials are relatively old and started patient accrual before 2000. (13, 20-23, 31) These patients might have had an in general lower life expectancy than in our cohort.

The largest trial to date comparing perioperative AI-based CTX with local treatment did not find an additional value of CTX in patients with STS. (13) However, in this trial 6% of the patients had a low-grade tumour and 24% had a tumour smaller than 5 cm (13), which in general are considered to be low-risk tumours. (2, 33) A recent post-hoc subgroup analysis of this trial showed a beneficial effect (HR 0.50) of AI-based CTX in a small subgroup of patients with a predicted 10-year OS of ≤ 60% based on the prognostic nomogram Sarculator. (4) In addition, another study found a 5-year OS of 66% in a subgroup of patients who received AI-based CTX

with a predicted 10-year OS of ≤ 60% based on the Sarculator. (6) These findings are comparable with our results, in which we found a HR of 0.66 for AI-based CTX with a 5-year OS of 70% in the AI-based CTX group and 59% in the no CTX group in the high PERSARC-risk population with a predicted 5-year OS of ≤ 66%.

This study did not find a beneficial effect of (AI-based) CTX on OS in low PERSARC-risk eSTS patients. These results are in line with previous studies that suggest that perioperative CTX should only be offered in a selected group of high-risk patients, as well as clinical guidelines that state that CTX is not standard of care, but could be considered in high-risk patients. (2, 4, 24, 26, 33-35). However, accurate identification of this high-risk subgroup remains unclear. Some studies suggested that patients with high-grade, large eSTS (≥ 8 or 10 cm) should receive CTX (24, 26, 34, 35), while other studies suggested that patients with a 10-year predicted OS <60% should be selected. (4, 6) Our study showed that patients with a predicted 5-year OS of <66% benefit of perioperative AI-based CTX. Although, this study showed that risk stratification with PERSARC might be useful for the identification of patients who benefit from perioperative AI-based CTX, with this study design we were unable to identify the optimal threshold when AI-based CTX is beneficial. In addition, this threshold could be varying across histological subtypes as some subtypes are more chemo sensitive than others. However, due to limited power we were unable to stratify our analyses for histological subtype.

In this study, we identified a subgroup of patients with high PERSARC-risk who benefit from AI-based CTX based on the PERSARC prediction tool. This prediction tool includes clinical parameters only and has an overall good discriminative ability (C-index: 0.68). (9) However, it might be that the identification of high-risk patients based on clinical parameters only is less adequate and that the prediction of CTX response might be improved by biological factors. (36, 37) A promising biomarker is the gene expression signature CINSARC, which showed to be a strong predictor for metastatic disease. (36, 38) The potential of this gene expression signature to identify high-risk patients that may benefit from CTX will be evaluated in future trials. (39)

This study has some weaknesses inherent to its retrospective and observational design. We acknowledge that there is a confounding by indication bias in all cohort studies in which the effectiveness of a treatment is assessed. Despite our effort to account for the most important confounders using weights (IPTW), the difference in OS between AI-based CTX and no CTX might also (partially) be explained by residual confounding due to unmeasured or not-fully-modelled explanatory covariates such as performance score. In addition, this study lacked the ability to capture additional relevant information about CTX dose, number of cycles, motivation for chemotherapy

administration and toxicity. Details about CTX regimen were available for 1259 out of 1635 patients of which 82% received AI-based CTX. Although these results imply that the standard practice of AI-based CTX was mainly used, we were unable to account for differences in CTX administration. Therefore, we performed a subgroup analysis including AI-based CTX only, excluding a considerable part of the patients of whom the CTX regimen was unknown. Furthermore, this study included EORTC data in which patients received 50 mg/m^2 doxorubicin and 5g/m^2 ifosfamide, which are both relatively low doses compared with the current most commonly used dose of 75 mg/m^2 doxorubicin and 9-10 g/m^2 ifosfamide. (40) Furthermore, in this study patients older than 70 years were excluded, as these patients rarely receive CTX. Therefore, the conclusions of this study may not be extrapolated to this age group.

Moreover, this study only included patients who were surgically treated. The starting point of this study was date of surgery rather than date of first treatment. This means that patients who received neoadjuvant CTX but did not receive surgery because of disease progression or death, were excluded. The consequent exclusion of patients failing to neoadjuvant CTX might have biased the results in favour of CTX. On the other hand, in patients who received neoadjuvant CTX (14.7% in this series), surgery is usually delayed with ±3 months because of the neoadjuvant chemotherapy. Therefore, patients who received neoadjuvant CTX with surgery had a delay of ±3 months before they received surgery compared to patients who received surgery alone. This might have resulted in an underestimation of the survival within the CTX group compared to the no CTX group.

Conclusion

In a selected group of eSTS patients with a high-risk profile (predicted 5-year OS ≤66%) based on the PERSARC prediction tool, perioperative AI-based CTX has a beneficial effect on OS with an absolute 5-year survival benefit of 11%. In concordance with the literature, we did not find a beneficial effect of all type CTX in the overall population of primary high-grade eSTS. Therefore, perioperative AI-based CTX should only be considered in predicted high-risk eSTS patients. Given the retrospective nature of this study, the findings should be independently, preferably prospectively, validated in a harm-benefit analysis.

References

1. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. European journal of cancer (Oxford, England : 1990). 2013;49(3):684-95.
2. Casali PG, Abecassis N, Aro HT, Bauer S, Biagini R, Bielack S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2018;29(Suppl 4):iv51-iv67.
3. Zagars GK, Ballo MT, Pisters PW, Pollock RE, Patel SR, Benjamin RS, et al. Prognostic factors for patients with localized soft-tissue sarcoma treated with conservation surgery and radiation therapy: an analysis of 1225 patients. Cancer. 2003;97(10):2530-43.
4. Pasquali S, Pizzamiglio S, Touati N, Litiere S, Marreaud S, Kasper B, et al. The impact of chemotherapy on survival of patients with extremity and trunk wall soft tissue sarcoma: revisiting the results of the EORTC-STBSG 62931 randomised trial. European journal of cancer (Oxford, England : 1990). 2019;109:51-60.
5. Gronchi A, Palmerini E, Quagliuolo V, Martin Broto J, Lopez Pousa A, Grignani G, et al. Neoadjuvant Chemotherapy in High-Risk Soft Tissue Sarcomas: Final Results of a Randomized Trial From Italian (ISG), Spanish (GEIS), French (FSG), and Polish (PSG) Sarcoma Groups. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2020;38(19):2178-86.
6. Pasquali S, Palmerini E, Quagliuolo V, Martin-Broto J, Lopez-Pousa A, Grignani G, et al. Neoadjuvant chemotherapy in high-risk soft tissue sarcomas: A Sarculator-based risk stratification analysis of the ISG-STS 1001 randomized trial. Cancer. 2022;128(1):85-93.
7. Benjamin RS. There is more to soft tissue sarcomas than just grade and size. Cancer. 2022;128(1):28-9.
8. Gamboa AC, Gronchi A, Cardona K. Soft-tissue sarcoma in adults: An update on the current state of histiotype-specific management in an era of personalized medicine. CA: a cancer journal for clinicians. 2020;70(3):200-29.
9. van Praag VM, Rueten-Budde AJ, Jeys LM, Laitinen MK, Pollock R, Aston W, et al. A prediction model for treatment decisions in high-grade extremity soft-tissue sarcomas: Personalised sarcoma care (PERSARC). European journal of cancer (Oxford, England : 1990). 2017;83:313-23.
10. Rueten-Budde AJ, van Praag VM, van de Sande MAJ, Fiocco M. Dynamic prediction of overall survival for patients with high-grade extremity soft tissue sarcoma. Surgical oncology. 2018;27(4):695-701.
11. Rueten-Budde AJ, van Praag VM, van de Sande MAJ, Fiocco M. External validation and adaptation of a dynamic prediction model for patients with high-grade extremity soft tissue sarcoma. Journal of surgical oncology. 2021;123(4):1050-6.
12. Kent DM, Paulus JK, van Klaveren D, D'Agostino R, Goodman S, Hayward R, et al. The Predictive Approaches to Treatment effect Heterogeneity (PATH) Statement. Ann Intern Med. 2020;172(1):35-45.
13. Woll PJ, Reichardt P, Le Cesne A, Bonvalot S, Azzarelli A, Hoekstra HJ, et al. Adjuvant chemotherapy with doxorubicin, ifosfamide, and lenograstim for resected soft-tissue sarcoma (EORTC 62931): a multicentre randomised controlled trial. The Lancet Oncology. 2012;13(10):1045-54.
14. Trojani M, Contesso G, Coindre JM, Rouesse J, Bui NB, de Mascarel A, et al. Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading system. Int J Cancer. 1984;33(1):37-42.

15. Acem I, Smit MM, Verhoef C, van Houdt WJ, Haas RL, van der Hage JA, et al. Management of Soft Tissue Sarcomas in Extremities: Variation in Treatment Recommendations and Surveillance According to Specialty and Continent. Annals of surgical oncology. 2021;28(12):7923-36.
16. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Controlled clinical trials. 1996;17(4):343-6.
17. Nieto FJ, Coresh J. Adjusting survival curves for confounders: a review and a new method. American journal of epidemiology. 1996;143(10):1059-68.
18. Rubin DB. Multiple Imputation After 18+ Years. Journal of the American Statistical Association. 1996;91(434):473-89.
19. R Development Core Team. R: A language and environment for statistical computing. 3.6.3 ed. Vienna, Austria: R Foundation for Statistical Computing; 2010.
20. Fakhrai N, Ebm C, Kostler WJ, Jantsch M, Abdolvahab F, Dominkus M, et al. Intensified adjuvant IFADIC chemotherapy in combination with radiotherapy versus radiotherapy alone for soft tissue sarcoma: long-term follow-up of a prospective randomized feasibility trial. Wiener klinische Wochenschrift. 2010;122(21-22):614-9.
21. Frustaci S, Gherlinzoni F, De Paoli A, Bonetti M, Azzarelli A, Comandone A, et al. Adjuvant chemotherapy for adult soft tissue sarcomas of the extremities and girdles: results of the Italian randomized cooperative trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(5):1238-47.
22. Gortzak E, Azzarelli A, Buesa J, Bramwell VH, van Coevorden F, van Geel AN, et al. A randomised phase II study on neo-adjuvant chemotherapy for 'high-risk' adult soft-tissue sarcoma. European journal of cancer (Oxford, England : 1990). 2001;37(9):1096-103.
23. Petrioli R, Coratti A, Correale P, D'Aniello C, Grimaldi L, Tanzini G, et al. Adjuvant epirubicin with or without Ifosfamide for adult soft-tissue sarcoma. Am J Clin Oncol. 2002;25(5):468-73.
24. Zaidi MY, Ethun CG, Tran TB, Poultsides G, Grignol VP, Howard JH, et al. Assessing the Role of Neoadjuvant Chemotherapy in Primary High-Risk Truncal/Extremity Soft Tissue Sarcomas: An Analysis of the Multi-institutional U.S. Sarcoma Collaborative. Annals of surgical oncology. 2019;26(11):3542-9.
25. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Impact of perioperative chemotherapy and radiotherapy in patients with primary extremity soft tissue sarcoma: retrospective analysis across major histological subtypes and major reference centres. European journal of cancer (Oxford, England : 1990). 2018;105:19-27.
26. Mullen JT, Kobayashi W, Wang JJ, Harmon DC, Choy E, Hornicek FJ, et al. Long-term follow-up of patients treated with neoadjuvant chemotherapy and radiotherapy for large, extremity soft tissue sarcomas. Cancer. 2012;118(15):3758-65.
27. Pervaiz N, Colterjohn N, Farrokhyar F, Tozer R, Figueredo A, Ghert M. A systematic meta-analysis of randomized controlled trials of adjuvant chemotherapy for localized resectable soft-tissue sarcoma. Cancer. 2008;113(3):573-81.
28. Istl AC, Ruck JM, Morris CD, Levin AS, Meyer CF, Johnston FM. Call for improved design and reporting in soft tissue sarcoma studies: A systematic review and meta-analysis of chemotherapy and survival outcomes in resectable STS. Journal of surgical oncology. 2019;119(7):824-35.
29. Graham DS, van Dams R, Jackson NJ, Onyshchenko M, Eckardt MA, DiPardo BJ, et al. Chemotherapy and Survival in Patients with Primary High-Grade Extremity and Trunk Soft Tissue Sarcoma. Cancers (Basel). 2020;12(9).
30. Gronchi A, Frustaci S, Mercuri M, Martin J, Lopez-Pousa A, Verderio P, et al. Short, full-dose adjuvant chemotherapy in high-risk adult soft tissue sarcomas: a randomized clinical trial from

the Italian Sarcoma Group and the Spanish Sarcoma Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30(8):850-6.
31. Issels RD, Lindner LH, Verweij J, Wessalowski R, Reichardt P, Wust P, et al. Effect of Neoadjuvant Chemotherapy Plus Regional Hyperthermia on Long-term Outcomes Among Patients With Localized High-Risk Soft Tissue Sarcoma: The EORTC 62961-ESHO 95 Randomized Clinical Trial. JAMA Oncol. 2018;4(4):483-92.
32. Davis EJ, Chugh R, Zhao L, Lucas DR, Biermann JS, Zalupski MM, et al. A randomised, open-label, phase II study of neo/adjuvant doxorubicin and ifosfamide versus gemcitabine and docetaxel in patients with localised, high-risk, soft tissue sarcoma. European journal of cancer (Oxford, England : 1990). 2015;51(13):1794-802.
33. Von Mehren M, Kane JM, Agulnik M, Bui MM, Ganjoo KN, George S, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. 2018;16(5):536-63.
34. Grobmyer SR, Maki RG, Demetri GD, Mazumdar M, Riedel E, Brennan MF, et al. Neo-adjuvant chemotherapy for primary high-grade extremity soft tissue sarcoma. Annals of oncology : official journal of the European Society for Medical Oncology. 2004;15(11):1667-72.
35. Mahmoud O, Tunceroglu A, Chokshi R, Benevenia J, Beebe K, Patterson F, et al. Overall survival advantage of chemotherapy and radiotherapy in the perioperative management of large extremity and trunk soft tissue sarcoma; a large database analysis. Radiother Oncol. 2017;124(2):277-84.
36. Chibon F, Lagarde P, Salas S, Pérot G, Brouste V, Tirode F, et al. Validated prediction of clinical outcome in sarcomas and multiple types of cancer on the basis of a gene expression signature related to genome complexity. Nature medicine. 2010;16(7):781-7.
37. Bertucci F, De Nonneville A, Finetti P, Perrot D, Nilbert M, Italiano A, et al. The Genomic Grade Index predicts postoperative clinical outcome in patients with soft-tissue sarcoma. Annals of oncology : official journal of the European Society for Medical Oncology. 2018;29(2):459-65.
38. Le Guellec S, Lesluyes T, Sarot E, Valle C, Filleron T, Rochaix P, et al. Validation of the Complexity INdex in SARComas prognostic signature on formalin-fixed, paraffin-embedded, soft-tissue sarcomas. Annals of oncology : official journal of the European Society for Medical Oncology. 2018;29(8):1828-35.
39. Filleron T, Le Guellec S, Chevreau C, Cabarrou B, Lesluyes T, Lodin S, et al. Value of peri-operative chemotherapy in patients with CINSARC high-risk localized grade 1 or 2 soft tissue sarcoma: study protocol of the target selection phase III CHIC-STS trial. BMC cancer. 2020;20(1):716.
40. Roland CL, van Houdt W, Gronchi A. The Landmark Series: Multimodality Treatment of Extremity Sarcoma. Annals of surgical oncology. 2020;27(10):3672-82.

Appendix

Appendix A. Methodology

Included centres

The included centres were Aarhus University Hospital (Aarhus, Denmark), Netherlands Cancer Institute (Amsterdam, the Netherlands), Haukeland University Hospital (Bergen, Norway), Helios Klinikum Berlin-Buch (Berlin, Germany), Royal Orthopaedic Hospital (Birmingham, UK), Sahlgrenska University Hospital (Gothenberg, Sweden), Medical University Graz (Graz, Austria), University Medical Centre Groningen (Groningen, the Netherlands), Nationwide cancer registry for bone and soft tissue tumours BSTT and JCOG304 study (Japan), Leiden University Medical Centre (Leiden, the Netherlands), Linköping University Hospital (Linköping, Sweden), The Royal Marsden (London and Surrey, UK), Skåne University Hospital (Lund, Sweden), Radboud Medical Centre (Nijmegen, the Netherlands), The Norwegian Radium Hospital (Oslo, Norway), Erasmus Medical Centre (Rotterdam, the Netherlands), Royal National Orthopaedic Hospital (Stanmore, UK), Karolinska University Hospital (Stockholm, Sweden), Mount Sinai Hospital (Toronto, Canada) and Umeå University Hospital (Umeå, Sweden) and the EORTC trial 62931.

Variable definitions

Tumour size was measured as the maximum diameter of tumour mass on imaging-techniques or based on pathological reports if preoperative imaging was not available. Depth was categorized as deep or superficial relative to the investing fascia. Surgical margin was categorized as 'R0' for negative margin and 'R1-R2' for a positive margin with tumour cells in the inked surface of the resection margin. (1) Histological subtypes were retrieved from pathology reports and were classified into 7 categories according to the World Health Organization (WHO) classification (2): leiomyosarcoma (LMS), liposarcoma (LPS), myxofibrosarcoma (MFS), undifferentiated pleomorphic sarcoma and (pleomorphic) STS not-otherwise-specified (UPS and NOS), malignant peripheral nerve sheath tumour (MPNST), synovial sarcoma (SS), spindle cell sarcoma and other. The "other" category included angiosarcoma, pleiomorphic rhabdomyosarcoma and other histological subtypes under-presented in our data.

Appendix B. Baseline characteristics for all CTX-regimens vs no CTX

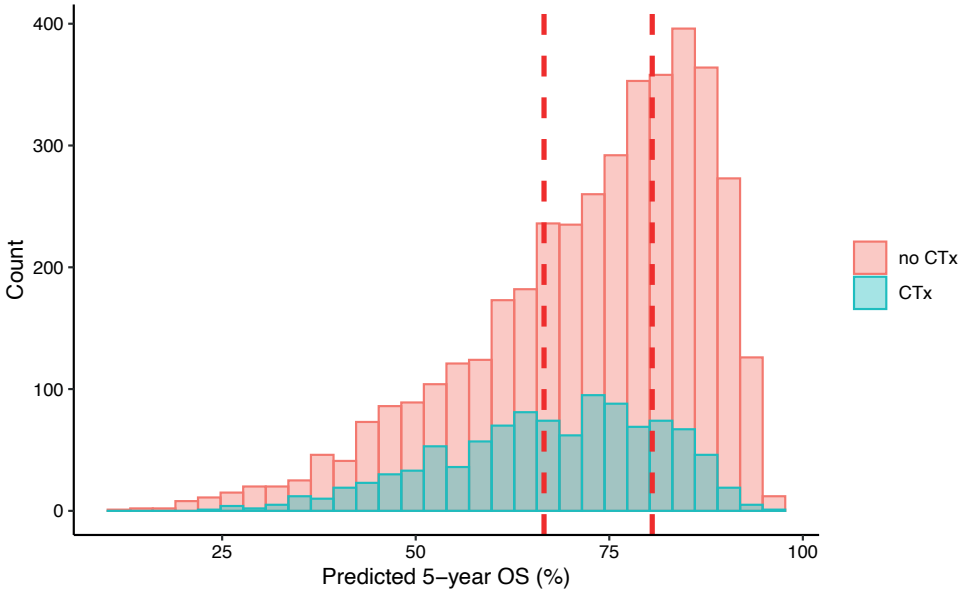

Figure 1. Predicted 5-year OS probability calculated by PERSARC prediction tool.
In pink: patients that did not receive chemotherapy.
In blue: patients who received chemotherapy. Dashed lines: 33% and 66% quantile.

Table 1. Patient characteristics per PERSARC-risk group

	High PERSARC-risk (N=1897)	Intermediate PERSARC-risk (N=1897)	Low PERSARC-risk (N=1889)	Overall (N=5683)
Baseline predicted 5-year OS (%)				
Mean (SD)	52.8 (10.4)	73.3 (4.07)	86.0 (3.82)	70.7 (15.3)
Median (IQR)	55.0 (46.6-61.2)	73.5 (69.9-76.9)	85.6 (82.9-88.9)	73.4 (61.2-82.9)
Chemotherapy				
No	1162 (61.3%)	1307 (68.9%)	1578 (83.6%)	4047 (71.2%)
Yes	735 (38.7%)	590 (31.1%)	310 (16.4%)	1635 (28.8%)
Missing	*0*	*0*	*1*	*1*
Chemotherapy (detailed)				
No CTX	1162 (61.8%)	1307 (69.2%)	1578 (83.7%)	4047 (71.5%)
Adjuvant	319 (17.0%)	287 (15.2%)	170 (9.0%)	776 (13.7%)
Neoadjuvant	159 (8.5%)	103 (5.4%)	50 (2.7%)	312 (5.5%)
Neo- and adjuvant	241 (12.8%)	193 (10.2%)	88 (4.7%)	522 (9.3%)
Missing	*16*	*7*	*3*	*26*
Age at surgery				
Mean (SD)	57.6 (11.3)	51.7 (13.4)	45.6 (13.3)	51.7 (13.6)
Median (IQR)	61 (52-66)	54 (43-63)	46 (36-57)	54 (42-63)
Size (cm)				
Mean (SD)	13.3 (5.59)	7.98 (4.17)	4.55 (2.58)	8.60 (5.60)
Median (IQR)	12.0 (9.0-16.0)	7.00 (5.0-10.0)	4.00 (3.0-6.0)	7.00 (4.5-11.0)
Missing	*90*	*119*	*89*	*298*
Depth				
Deep	1647 (91.6%)	1350 (74.9%)	905 (50.9%)	3902 (72.6%)
Superficial	151 (8.4%)	453 (25.1%)	872 (49.1%)	1476 (27.4%)
Missing	*99*	*94*	*112*	*305*
Histological subtype				
LMS	162 (8.5%)	185 (9.8%)	242 (12.8%)	589 (10.4%)
LPS	242 (12.8%)	428 (22.6%)	510 (27.0%)	1180 (20.8%)
MFS	231 (12.2%)	246 (13.0%)	309 (16.4%)	786 (13.8%)
UPS and NOS	671 (35.4%)	521 (27.5%)	323 (17.1%)	1515 (26.7%)
MPNST	207 (10.9%)	114 (6.0%)	47 (2.5%)	368 (6.5%)
SS	230 (12.1%)	246 (13.0%)	255 (13.5%)	731 (12.9%)
Other	154 (8.1%)	156 (8.2%)	202 (10.7%)	512 (9.0%)
Missing	*0*	*1*	*1*	*2*
Grade				
2	62 (3.3%)	205 (10.8%)	580 (30.7%)	847 (14.9%)
3	860 (45.3%)	688 (36.3%)	485 (25.7%)	2033 (35.8%)

	High PERSARC-risk (N=1897)	Intermediate PERSARC-risk (N=1897)	Low PERSARC-risk (N=1889)	Overall (N=5683)
High grade not further specified	975 (51.4%)	1004 (52.9%)	824 (43.6%)	2803 (49.3%)
Surgical margin				
R0	1654 (89.2%)	1722 (92.8%)	1690 (93.2%)	5066 (91.7%)
R1-R2	201 (10.8%)	133 (7.2%)	123 (6.8%)	457 (8.3%)
Missing	*42*	*42*	*76*	*160*
Radiotherapy				
No RTX	1099 (58.4%)	1030 (54.6%)	897 (47.8%)	3026 (53.6%)
Adjuvant	542 (28.8%)	549 (29.1%)	583 (31.1%)	1674 (29.7%)
Neoadjuvant	235 (12.5%)	299 (15.9%)	389 (20.7%)	923 (16.4%)
Neo- and adjuvant	7 (0.4%)	8 (0.4%)	6 (0.3%)	21 (0.6%)
Missing	*14*	*11*	*14*	*39*

OS: overall survival, SD: standard deviation, IQR: interquartile range, LMS: leiomyosarcoma, LPS: liposarcoma, MFS: myxofibrosarcoma, UPS: undifferentiated pleomorphic sarcoma, NOS: (pleomorphic) soft tissue sarcomas not otherwise specified, MPNST: malignant peripheral nerve sheet tumour, SS: Synovial sarcoma, CTX: chemotherapy, RTX: radiotherapy.

Appendix C. Primary endpoint: Overall survival for all CTX regimens vs no CTX

Logistic regression models with chemotherapy as outcome variable were estimated for the overall population and each PERSARC-risk group to create weights for the weighted Kaplan-Meier analyses. The logistic regression model for the overall population and per PERSARC-risk group is displayed in **Table 1**. **Figure 1** displays the distribution of the weights based on the models in **Table 1.**

Table 1. Logistic regression model with outcome chemotherapy treatment in the overall population and per PERSARC-risk group

Variable	Overall OR (95% CI)	High OR (95% CI)	Intermediate OR (95% CI)	Low OR (95% CI)
Age (ref: 0 years, per 1 year)	0.975 (0.970 – 0.980)	0.950 (0.940 – 0.960)	0.960 (0.949 – 0.970)	0.972 (0.960 – 0.983)
Tumour size (ref: 0 cm, per 1 cm)	1.06 (1.04 – 1.07)	0.998 (0.977 – 1.02)	0.979 (0.937 – 1.02)	1.08 (1.01 – 1.15)
Tumour depth				
Deep	1	1	1	1
Superficial	0.385 (0.322 – 0.461)	0.642 (0.433 – 0.951)	0.492 (0.360 – 0.672)	0.521 (0.385 – 0.707)
Histology				
LMS	1	1	1	1
LPS	0.677 (0.528 – 0.868)	0.665 (0.431 – 1.03)	0.850 (0.545 – 1.33)	1.28 (0.745 – 2.20)
Myxofibrosarcoma	0.510 (0.386 – 0.676)	0.393 (0.249 – 0.620)	0.641 (0.404 – 1.02)	0.765 (0.419 – 1.40)
UPS and NOS	0.827 (0.652 – 1.05)	0.784 (0.544 – 1.13)	0.683 (0.460 – 1.01)	0.788 (0.446 – 1.39)
MPNST	0.495 (0.357 – 0.687)	0.322 (0.201 – 0.515)	0.249 (0.136 – 0.457)	0.755 (0.281 – 2.03)
SS	1.20 (0.911 – 1.57)	0.718 (0.457 – 1.13)	0.804 (0.505 – 1.28)	2.14 (1.25 – 3.65)
Other	0.582 (0.433 –0.780)	0.449 (0.276 – 0.728)	0.422 (0.250 – 0.709)	1.02 (0.571 – 1.81)
Grade				
2	1	1	1	1
3	2.13 (1.57 – 2.89)	1.48 (0.873 – 2.50)	1.55 (1.01 – 2.39)	2.13 (1.41 – 3.20)
Margin				
R0	1	1	1	1
R1-R2	0.486 (0.369 – 0.640)	0.478 (0.329 – 0.693)	0.336 (0.193 – 0.585)	0.674 (0.351 – 1.29)
Radiotherapy				
No RTX	1	1	1	1
Adjuvant	0.685 (0.592 – 0.793)	0.677 (0.531 – 0.863)	1.03 (0.782 – 1.35)	0.823 (0.596 – 1.14)
Neoadjuvant	0.339 (0.275 – 0.419)	0.519 (0.372 – 0.722)	0.468 (0.329 – 0.667)	0.240 (0.149 – 0.386)

OR: odds ratio, CI: confidence interval, LMS: leiomyosarcoma, LPS: liposarcoma, MFS: myxofibrosarcoma, UPS: undifferentiated pleomorphic sarcoma, NOS: (pleomorphic) soft tissue sarcomas not-otherwise-specified, MPNST: malignant peripheral nerve sheet tumour, SS: synovial sarcoma, RTX: radiotherapy

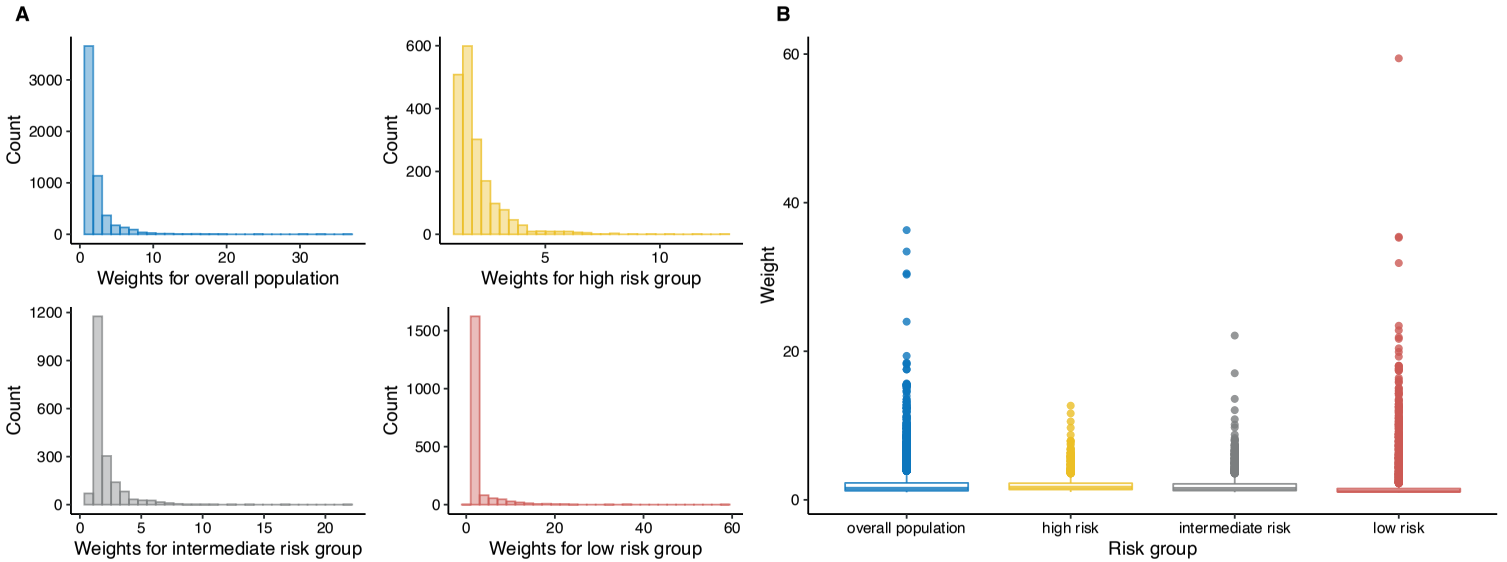

Figure 1A. Histograms of weights for overall population and within PERSARC-risk groups **B.** Boxplots of weights for overall population and within PERSARC-risk groups

Figure 1A and **1B** show that the weights are mainly concentrated ≤2 with a few outliers.

Appendix D. Baseline characteristics for anthracycline and ifosfamide-based chemotherapy regimen only
Table 1. Patient characteristics per PERSARC-risk group

	High PERSARC-risk (N=1597)	Intermediate PERSARC-risk (N=1689)	Low PERSARC-risk (N=1798)	Overall (N=5084)
Baseline predicted 5-year OS (%)				
Mean (SD)	52.9 (10.3)	73.3 (4.08)	86.0 (3.82)	71.4 (15.1)
Chemotherapy				
No	1159 (72.6%)	1310 (77.6%)	1579 (87.8%)	4048 (79.6%)
Yes	438 (27.4%)	379 (22.4%)	219 (12.2%)	1036 (20.4%)
Chemotherapy (detailed)				
No CTX	1159 (72.6%)	1310 (77.6%)	1579 (87.8%)	4048 (79.6%)
Adjuvant	141 (8.8%)	155 (9.2%)	114 (6.3%)	410 (8.1%)
Neoadjuvant	89 (5.6%)	60 (3.6%)	32 (1.8%)	181 (3.6%)
Neo- and adjuvant	208 (13.0%)	164 (9.7%)	73 (4.1%)	445 (8.8%)
Age at surgery				
Mean (SD)	58.1 (11.0)	52.4 (13.2)	45.9 (13.2)	51.9 (13.5)
Size (cm)				
Mean (SD)	13.2 (5.73)	7.98 (4.29)	4.55 (2.60)	8.41 (5.61)
Missing	*83*	*109*	*85*	*277*
Depth				
Deep	1434 (91.9%)	1209 (74.2%)	861 (50.8%)	3504 (71.7%)
Superficial	126 (8.1%)	421 (25.8%)	834 (49.2%)	1381 (28.3%)
Missing	*37*	*59*	*103*	*199*
Histological subtype				
LMS	135 (8.5%)	171 (10.1%)	235 (13.1%)	541 (10.6%)
LPS	216 (13.5%)	387 (22.9%)	490 (27.3%)	1093 (21.5%)
MFS	197 (12.3%)	210 (12.4%)	291 (16.2%)	698 (13.7%)
UPS and NOS	550 (34.4%)	473 (28.0%)	316 (17.6%)	1339 (26.3%)
MPNST	171 (10.7%)	107 (6.3%)	46 (2.6%)	324 (6.4%)
SS	195 (12.2%)	203 (12.0%)	232 (12.9%)	630 (12.4%)
Other	133 (8.3%)	138 (8.2%)	187 (10.4%)	458 (9.0%)
Missing	*0*	*0*	*1*	*1*

	High PERSARC-risk (N=1597)	Intermediate PERSARC-risk (N=1689)	Low PERSARC-risk (N=1798)	Overall (N=5084)
Grade				
2	59 (8.1%)	198 (25.5%)	564 (55.7%)	821 (32.6%)
3	669 (91.9%)	578 (74.5%)	449 (44.3%)	1696 (67.4%)
Missing	*869*	*913*	*785*	*2567*
Margin				
R0	1396 (89.1%)	1536 (92.5%)	1613 (93.3%)	4545 (91.7%)
R1-R2	171 (10.9%)	124 (7.5%)	116 (6.7%)	411 (8.3%)
Missing	*30*	*29*	*69*	*128*
Radiotherapy				
No RTX	940 (59.0%)	931 (55.3%)	862 (48.3%)	2733 (54.0%)
Adjuvant	441 (27.7%)	468 (27.8%)	539 (30.2%)	1448 (28.6%)
Neoadjuvant	206 (12.9%)	277 (16.8%)	380 (21.3%)	863 (17.0%)
Neo- and adjuvant	6 (0.4%)	8 (0.5%)	5 (0.3%)	19 (0.4%)
Missing	*4*	*5*	*12*	*21*

OS: overall survival, SD: standard deviation, IQR: interquartile range, LMS: leiomyosarcoma, LPS: liposarcoma, MFS: myxofibrosarcoma, UPS: undifferentiated pleomorphic sarcoma, NOS: (pleomorphic) soft tissue sarcomas not-otherwise-specified, MPNST: malignant peripheral nerve sheet tumour, SS: Synovial sarcoma, CTX: chemotherapy, RTX: radiotherapy

Appendix E. Primary outcome: Overall survival for AI-based CTX vs no CTX

Logistic regression models with AI-based CTX vs no CTX as outcome were estimated for the overall population and for each PERSARC-risk group (high, intermediate, and low PERSARC-risk group) to create weights for the weighted Kaplan-Meier analyses. The logistic regression model for the overall population and per PERSARC-risk group is displayed in **Table 1**. **Figure 1** displays the distribution of the weights based on the models in **Table 1**.

Table 1. Logistic regression model with outcome AI-based chemotherapy treatment in the overall population and per PERSARC-risk group

Variable	Overall OR (95% CI)	High OR (95% CI)	Intermediate OR (95% CI)	Low OR (95% CI)
Age (ref: 0 years, per 1 year)	0.973 (0.967 - 0.979)	0.948 (0.936 – 0.960)	0.962 (0.950 – 0.974)	0.973 (0.959 – 0.986)
Tumour size (ref: 0 cm, per 1 cm)	1.05 (1.03 - 1.06)	0.992 (0.967 – 1.02)	0.987 (0.939 – 1.04)	1.09 (1.01 – 1.18)
Tumour depth				
Deep	1	1	1	1
Superficial	0.300 (0.241 – 0.372)	0.325 (0.182 – 0.581)	0.395 (0.273 – 0.570)	0.416 (0.292 – 0.594)
Histology				
LMS	1	1	1	1
LPS	0.667 (0.504 – 0.883)	0.705 (0.429 – 1.16)	0.713 (0.432 – 1.18)	1.21 (0.650 – 2.24)
Myxofibrosarcoma	0.293 (0.202 - 0.425)	0.277 (0.153 – 0.503)	0.331 (0.183 – 0.600)	0.350 (0.146 – 0.834)
UPS and NOS	0.745 (0.565 – 0.982)	0.726 (0.475 – 1.11)	0.578 (0.369 – 0.906)	0.909 (0.478 – 1.73)
MPNST	0.382 (0.255 - 0.572)	0.218 (0.120 – 0.396)	0.244 (0.122 – 0.489)	0.980 (0.329 – 2.92)
SS	1.19 (0.868 – 1.63)	0.777 (0.465 – 1.30)	0.719 (0.423 – 1.22)	2.28 (1.23 – 4.23)
Other	0.474 (0.334 – 0.672)	0.434 (0.243 – 0.774)	0.321 (0.175 – 0.589)	0.789 (0.397 – 1.57)
Grade				
2	1	1	1	1
3	1.72 (1.21 – 3.43)	1.17 (0.604 – 2.28)	1.29 (0.802 – 2.07)	1.92 (1.19 – 3.09)
Margin				
R0	1	1	1	1
R1-R2	0.324 (0.217 - 0.484)	0.314 (0.178 – 0.554)	0.255 (0.120 – 0.540)	0.397 (0.155 – 1.02)
Radiotherapy				
No RTX	1	1	1	1
Adjuvant	0.477 (0.399 - 0.572)	0.444 (0.323 – 0.609)	0.710 (0.513 – 0.984)	0.545 (0.371 – 0.802)
Neoadjuvant	0.269 (0.209 - 0.347)	0.415 (0.273 – 0.628)	0.389 (0.256 – 0.592)	0.162 (0.0913 – 0.289)

OR: odds ratio, CI: confidence interval, LMS: leiomyosarcoma, LPS: liposarcoma, MFS: myxofibrosarcoma, UPS: undifferentiated pleomorphic sarcoma, NOS: (pleomorphic) soft tissue sarcomas not-otherwise-specified, MPNST: malignant peripheral nerve sheet tumour, SS: synovial sarcoma, RTX: radiotherapy

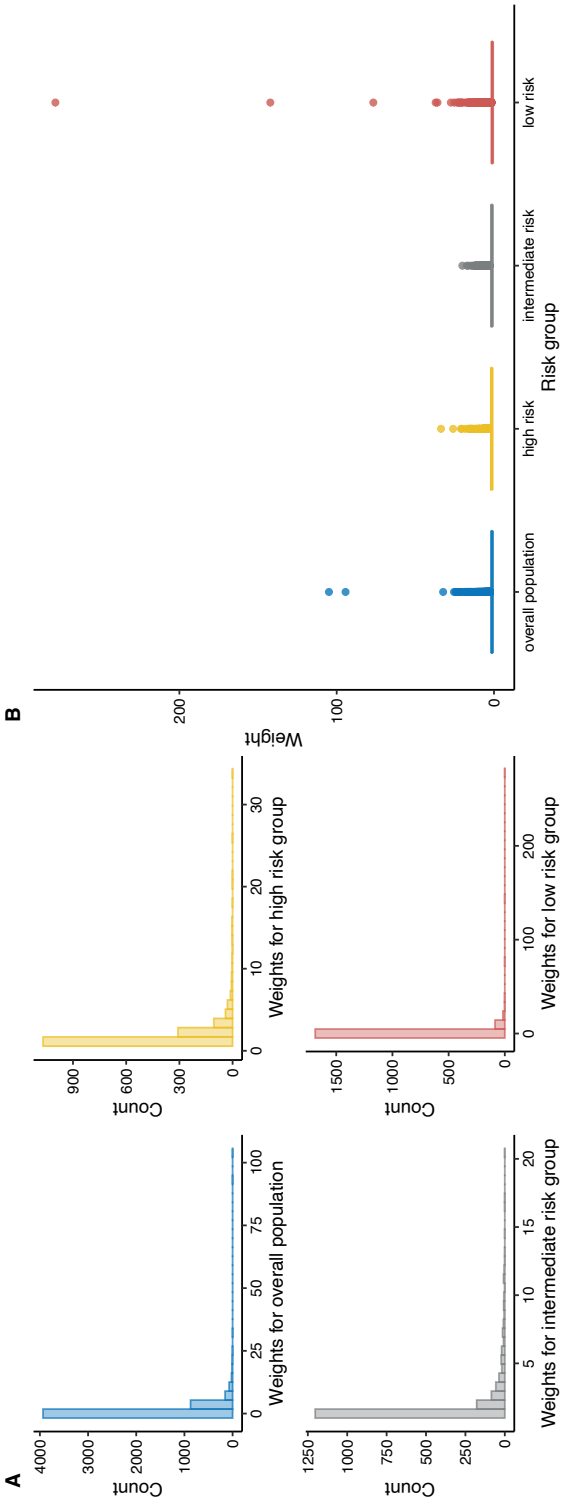

Figure 1A. Histograms of weights for overall population and within PERSARC-risk groups for AI-based CTX vs no CTX. **B.** Boxplots of weights for overall population and within PERSARC-risk groups for AI-based CTX vs no CT

References

1. Gundle KR, Kafchinski L, Gupta S, Griffin AM, Dickson BC, Chung PW, et al. Analysis of Margin Classification Systems for Assessing the Risk of Local Recurrence After Soft Tissue Sarcoma Resection. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(7):704-9.
2. WHO Classification of Tumours: Soft Tissue and Bone Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020.

Chapter 10

The Added Value of Chest Imaging After Neoadjuvant Radiotherapy for Soft Tissue Sarcoma of the Extremities and Trunk Wall: A Retrospective Cohort Study

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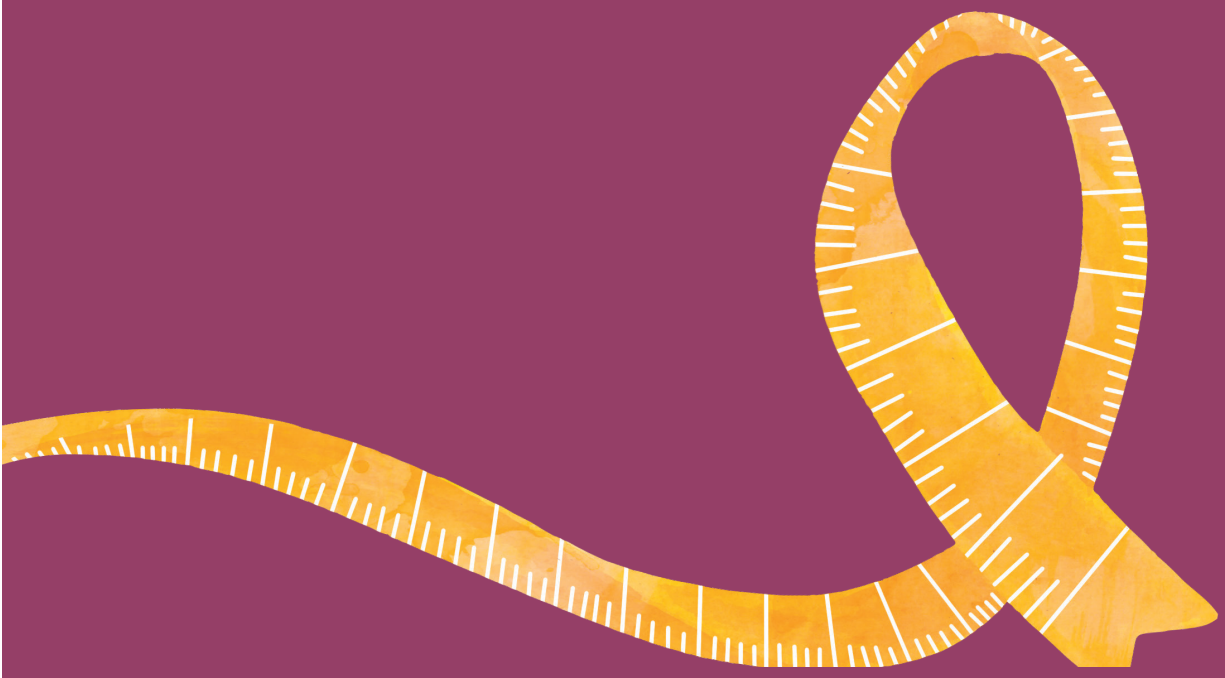

Abstract

Introduction

There is no clear evidence regarding the benefit of restaging for distant metastases after neoadjuvant radiotherapy (RTX) in patients with soft tissue sarcoma (STS) of the extremities and trunk wall. This study aimed to determine how often restaging of the chest identified metastatic disease that altered management in these patients.

Methods

We performed a single-centre retrospective study from 2010 to 2020. All patients with non-metastatic STS of the extremities and trunk wall who were treated with neoadjuvant RTX and received a staging and restaging chest CT scan or X-ray for distant metastasis were included. The outcome of interest was change in treatment strategy due to restaging after neoadjuvant RTX.

Results

Within the 144 patients who were staged and treated with neoadjuvant RTX, a restaging chest CT or X-ray was performed in 134 patients (93%). A change in treatment strategy due to new findings at restaging after RTX was observed in 26 out of 134 patients (19%). In 24 patients the scheduled resection of the primary STS was cancelled at restaging (24/134, 18%), given the findings at restaging. The other two patients did receive the intended local resection, but either with palliative intent, or as a part of a previously unplanned multimodality treatment.

Conclusion

In approximately one in five patients restaging results in a change in treatment strategy. This underlines the added value of routine restaging for distant metastases with chest CT or X-ray after neoadjuvant RTX in patients with STS.

Introduction

Approximately 30% of the patients with primary high-grade soft tissue sarcoma (STS) develop metastatic disease within 5 years after diagnosis or primary treatment. (1-3) In addition, 7-14% of the STS patients have distant metastases at presentation. (4, 5) STS mainly metastasize to the lungs. (6-8) Median time to pulmonary metastasis is around 11 months. (8, 9) Extrapulmonary metastases seem to occur later in time (median 22 months). (8) Metastatic STS is usually treated in a palliative setting. Especially patients with a metastatic-free interval < 1 year are treated palliatively as they have a poor prognosis. (7, 10-14) In this metastatic setting, the right balance between life expectancy and quality of life is considered to be very important.

Therefore, patients with primary sarcoma are usually staged with a Computed Tomography (CT) scan of the chest and/or abdomen to rule out distant metastases. (10, 15) If no metastases are found, patients are usually treated surgically with curative intent. (10, 15) (Neo)adjuvant radiotherapy (RTX) is typically indicated after multidisciplinary discussion in high-grade lesions considering risk factors for local recurrence, anticipated surgical margins, tumour size, grade and histological subtype. (10, 15, 16) Historically RTX was mainly delivered postoperatively. However, over the last few years a shift has occurred from adjuvant to neoadjuvant RTX. (17) The oncological outcomes between neoadjuvant and adjuvant RTX are comparable, but neoadjuvant RTX results in less long-term morbidity due to fibrosis, oedema and joint stiffness. (16, 18-20) The higher short-term wound complications of neoadjuvant RTX are often well managed in a specialised sarcoma centre or prevented with the use of reconstructive surgery. (16, 18-20) Due to the shift to neoadjuvant RTX, surgery is usually delayed with 12-15 weeks. Therefore, there has been an increasing interest in the need to accurately assess disease progression after neoadjuvant therapy.

Taking into consideration that the median time to pulmonary metastasis is 11 months, that patients with a metastatic-free interval of <1 year have a worse prognosis, and that patients with metastatic disease are treated differently, restaging after neoadjuvant RTX could influence the planned treatment strategy if formerly non-detectable distant metastasis appear in the time between staging and definitive surgery. However, to our best knowledge, restaging for distant disease is not standard practice in multiple sarcoma centres across Europe, and none of the current international clinical guidelines (European Society for Medical Oncology [ESMO] and National Comprehensive Cancer Network [NCCN]) have incorporated restaging for distant disease in their recommendations. (10, 15) Furthermore, there are no studies in STS that support the added value of restaging chest CT or X-ray. Therefore, the aim of this study was to assess the value of distant restaging with chest CT or X-ray after neoadjuvant RTX by

determining how often restaging identified metastatic disease that altered treatment management in patients with localized STS of the extremity and trunk wall.

Methods

Study design

Patients with localized STS of the extremities or trunk from a tertiary referral centre in The Netherlands (Erasmus MC Cancer Institute) were included in this retrospective single centre cohort study. This study was approved by the local Ethical Committee. Patients were identified from the centre's pathology database and from the radiotherapy department. The inclusion period was from January 2010 until December 2020.

The primary outcome of interest was change in treatment strategy after restaging chest CT or chest X-ray for distant metastases.

Study population

Adults (≥ 18 years) with histologically proven STS of the extremity or trunk wall treated with neoadjuvant RTX with curative intent who received a staging CT or X-ray of the chest at presentation and after RTX were included in this study. Patients were excluded if they had synchronous distant metastases, received neoadjuvant CTX or ILP, had a Kaposi's sarcoma or alveolar rhabdomyosarcoma, or if they had a concurrent primary malignancy at staging.

Study procedure

All patients received the standard work-up for soft tissue sarcoma that included an MRI scan of the primary site for local staging, and a CT scan (or X-ray) of the chest (and abdomen) for distant staging. All diagnoses were assessed by a specialized sarcoma pathologist according to the WHO classification. (21) All newly diagnosed patients were discussed during the multidisciplinary tumour board (MDT) meetings consisting of dedicated surgical oncologists, medical oncologists, radiation oncologists, radiologists and pathologists.

All patients within our centre were treated in accordance with the ESMO guidelines. (10) RTX was preferably delivered in the preoperative setting in our centre within the study period. This treatment generally consisted of long-course RTX with a total dose of 50 Gy delivered in 25 fractions of 2 Gy in 5 weeks. RTX was followed by surgery after ±10 weeks.

Data collection

To investigate the value of restaging after neoadjuvant RTX, patients and tumour characteristics, staging and restaging imaging findings before and after neoadjuvant RTX, the planned treatment before RTX and the ultimate treatment after restaging, and the intention of the treatment (curative/palliative) were collected. A detailed description of the definitions used in this study for each variable can be found in **Appendix A**.

Staging and restaging

Staging chest CT or X-ray was defined as a chest CT, chest-abdomen CT, or chest X-ray made before neoadjuvant RTX at either the referring hospital or at our institution. Restaging chest CT or X-ray was defined as a chest CT, chest-abdomen CT, or chest X-ray made in the period between the last week of RTX administration and surgery, or start of any other treatment, or within 3 months after RTX if no additional treatment was offered.

All staging and restaging images were assessed by dedicated radiologists and discussed in sarcoma MDTs. The reports from the radiologists and the MDTs were retrospectively evaluated. The findings at staging and restaging were classified as not suspected, indeterminate or metastases. A detailed description for the classification of lesions found at staging and restaging can be found in **Appendix B**.

Statistical analysis

Descriptive statistics were used for the analyses of the data. Patient demographics, baseline characteristics and all outcomes were described with numbers and percentages for categorical variables and means with standard deviations (SD) or medians with interquartile ranges (IQR) for continuous variables. All analyses were performed in the statistical program R, version 4.0.5. (22)

Results

After removal of the duplicates from the pathology and radiotherapy database, 1061 patients with STS of the trunk wall and extremity were eligible. A total of 927 patients did not meet the selection criteria, resulting in 134 patients who were included in this study. Ten patients were excluded in this analysis since they did not receive a restaging chest CT/X-ray after neoadjuvant RTX. **Figure 1** depicts the flow diagram of patient selection.

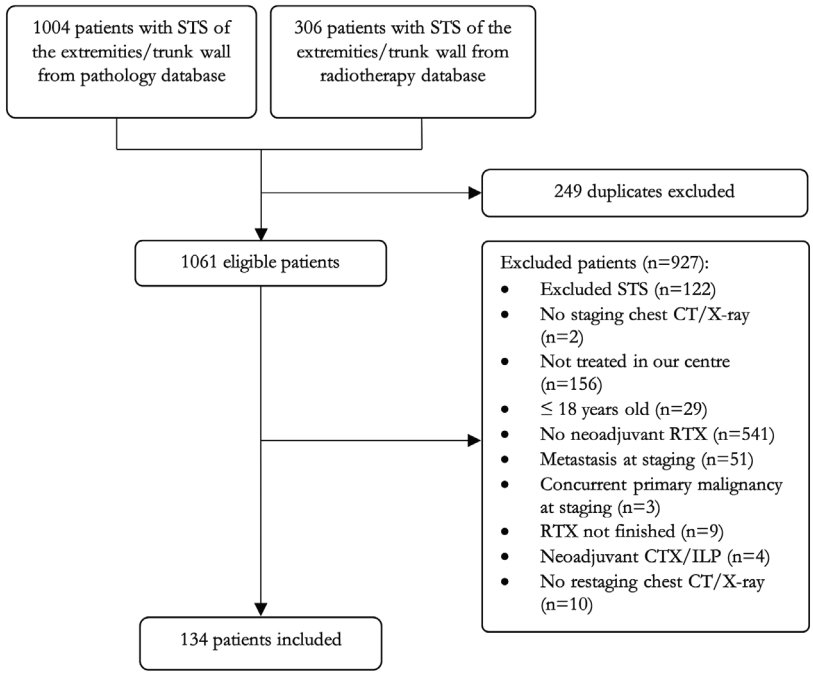

Figure 1. Study flow diagram

Median time between the first staging scan and start of RTX was 4.9 weeks (IQR 3.8-6.78). The median time between start of RTX and restaging was 9.4 weeks (IQR 8.9-10.4) (**Figure 2**).

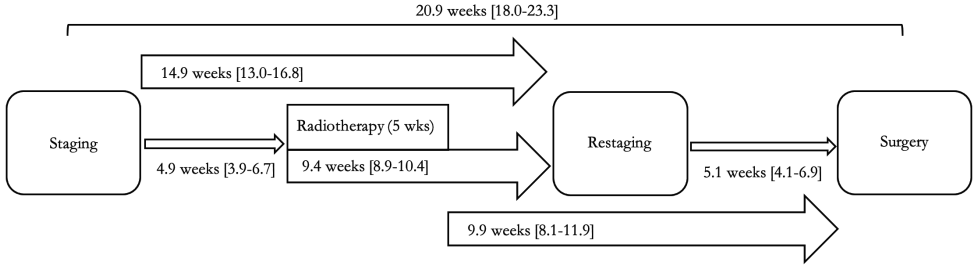

Figure 2. Timeline. Median time [IQR]

Imaging techniques

Primary staging for distant disease with a CT scan was performed in 131/134 patients (98%). The other 3 patients were staged with a chest X-ray (2%, 3/134). One hundred thirty patients were restaged with a CT scan (130/134, 97%). Four patients were restaged with a chest X-ray (3%, 4/134). The median age of the study population was 66 [IQR 52-74]. Baseline characteristics are depicted in **Table 1**.

Table 1. Baseline characteristics

	Overall (N=134)
Sex	
Female	53 (39.6%)
Male	81 (60.4%)
Age (years)	
Median [IQR]	66 [52-74]
ASA physical status	
ASA 1	25 (22.1%)
ASA 2	54 (47.8%)
ASA 3	31 (27.4%)
ASA 4	3 (2.7%)
Missing	*21*
Presentation	
Primary disease	124 (92.5%)
Recurrent disease	10 (7.5%)
Staging modality of chest	
X-ray	3 (2.2%)
Chest CT	78 (58.2%)
Chest/abdomen CT	53 (39.6%)
Restaging modality of chest	
X-ray	4 (3.0%)
Chest CT	94 (70.1%)
Chest/abdomen CT	36 (26.9%)
Size (mm)	
Median [IQR]	89 [61-130]
Missing	*9*
Histological subtype	
LMS	12 (9.0%)
MPNST	4 (3.0%)
MFS	28 (20.9%)
SS	3 (2.2%)
Other	7 (5.2%)
LPS	31 (23.1%)
UPS and NOS	49 (36.6%)
Grade	
Low grade	3 (3.0%)
High grade	96 (97.0%)
Missing	*35*
Depth	
Superficial	8 (6.0%)
Deep	126 (94.0%)

IQR: interquartile range, CT: computed tomography, mm: millimetres, LMS: leiomyosarcoma, MPNST: malignant peripheral nerve sheath tumour, MFS: myxofibrosarcoma, SS: synovial sarcoma, LPS: liposarcoma, UPS: undifferentiated pleomorphic sarcoma, NOS: soft tissue sarcoma – not otherwise specified

Imaging findings

Of the 134 patients who were restaged after neoadjuvant RTX, 91 patients did not have a suspected lesion at restaging (91/134, 68%). Twenty-four out of 134 patients (24/134, 18%) had metastases at restaging and 19 out of 134 patients (19/134, 14%) had an indeterminate lesion for which additional diagnostic tests or surveillance was needed (**Figure 3**).

Restaging findings after unsuspected staging CT/X-ray

Of the 96 patients with an unsuspected staging scan, 20 patients had newly emerging lesions on the restaging scan after neoadjuvant RTX (20/96, 21%). Fifteen patients had lesions suspected for metastases (15/96, 16%) and 5 patients had indeterminate lesions at restaging (5/96, 5%) (**Figure 3**). One patient with a newly emerging indeterminate lesion in the liver at restaging turned out to have a cholangiocarcinoma. Only two patients with a lesion suspected for metastasis received a biopsy after restaging which confirmed distant metastases (1 lung metastasis, 1 retroperitoneal metastasis both found on CT-thorax/abdomen).

Restaging findings after indeterminate lesions at staging CT/X-ray

Of the 38 patients with indeterminate lesions at staging, 9 patients had metastases at restaging (9/38, 24%) (none were confirmed by biopsy) (**Figure 3**). Fourteen patients still had an indeterminate lesion at restaging, which means that the pre-existing lesion did not decrease in number and in size and did not show an obvious progression in number and or in size, or that there were no newly developed nodules suspected for metastases. One patient with an indeterminate lesion in the pancreas at restaging turned out to have an adenocarcinoma in the head of the pancreas.

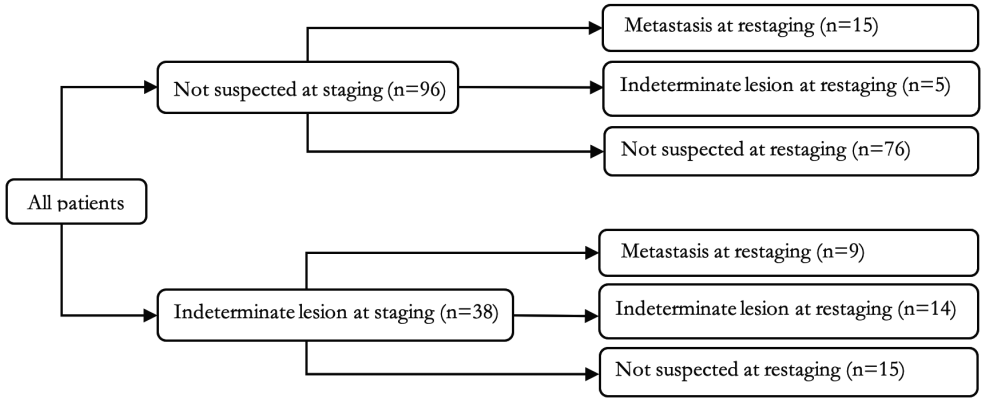

Figure 3. Findings on staging and restaging chest CT/X-ray

Change in strategy

Of the 134 patients who received a restaging chest CT scan or X-ray, 26 patients had a change in treatment strategy due to findings at restaging (19%). In 24 patients, resection of the primary STS was not indicated due to the findings at restaging (24/134, 18%).

Treatment of patients with metastatic lesions at restaging

Twenty-four patients were newly diagnosed with metastatic disease at restaging with CT scan or X-ray (24/134, 18%). Due to the metastatic findings, the treatment strategy was adjusted for 23 out of 24 patients (96%). The treatment intention changed for 22 out of 24 patients (92%). The majority of patients received best supportive care (9/24, 38%), or palliative chemotherapy (9/24, 38%) after the metastatic findings. Three out of 24 patients received multimodality treatment with chemotherapy and surgery for the primary tumour and/or distant metastases. One patient received multimodality treatment with curative intent. Two patients received multimodality treatment because of pain caused by the primary tumour or the distant metastases (**Table 2**).

One patient had two newly developed nodules of <5 mm at restaging which were classified in de radiology report as suspected of metastases. This is in accordance with the criteria in **Figure 1 of Appendix B**. Nevertheless, this patient received local surgery with curative intent. After surgery, follow-up CT scans revealed progression of the lung lesions to ≥10 mm and newly developed lung nodules.

Treatment of patients with indeterminate lesions at restaging

Of the 19 patients with indeterminate lesions at restaging, 17 patients had no change in treatment strategy. In two patients the indeterminate lesion turned out to be another primary malignancy after additional diagnostics (cholangiocarcinoma and adenocarcinoma in the head of the pancreas, respectively). Both patients did not receive any treatment for the STS after the finding (**Table 2**).

Table 2. Diagnostic findings of restaging after radiotherapy and change in treatment strategy

Staging	Restaging	N	Treatment	Curative intention	Change in treatment strategy	N (%)
Not Suspected	Not Suspected	75				
			Curative surgery	Yes	No	75 (100%)
Not Suspected	Indeterminate	5				
			Curative surgery	Yes	No	4 (80%)
			No treatment [a]	No	Yes	1 (20%)
Not Suspected	Metastases	15				
			No treatment	No	Yes	6 (40%)
			Local surgery	Yes	No	1 (7%)
			Palliative chemotherapy	No	Yes	5 (33%)
			Palliative chemotherapy + metastasectomy lymph nodes inguinal	No	Yes	1 (7%)
			Chemotherapy + local surgery + metastasectomy solidary retroperitoneal metastasis	Yes	Yes	1 (7%)
			Palliative intent – treatment unknown	No	Yes	1 (7%)
Indeterminate	Not Suspected	15				
			Curative surgery	Yes	No	15 (100%)
Indeterminate	Indeterminate	14				
			No treatment [b]	No	Yes	1 (7%)
			Curative surgery	Yes	No	13 (93%)
Indeterminate	Metastases	9				
			No treatment	No	Yes	3 (33%)
			Palliative chemotherapy + local surgery	No	Yes	1 (11%)
			Palliative chemotherapy	No	Yes	4 (44%)
			Palliative intent – treatment unknown	No	Yes	1 (11%)

[a] not due to STS, but due to cholangiocarcinoma found at restaging

[b] not due to STS, but due to pancreatic cancer found at restaging

Discussion

We evaluated the value of restaging with chest imaging for distant metastases after neoadjuvant RTX in patients with localized soft tissue sarcoma of the extremities and

trunk wall. The study showed a change in treatment strategy in 19% of the patients due to new findings at restaging after neoadjuvant RTX. In 18% of the patients the intended local resection of the primary tumour was not indicated after findings at restaging.

Local staging of STS has important implications for the choice of optimal treatment. Local control could be improved with (neo)adjuvant RTX in patients with large, high-grade, deep-seated tumours if a compartmental resection is not indicated. (10, 16) Distant staging has important implications on treatment options and intention of treatment. International clinical guidelines recommend screening for distant metastases by staging patients with contrast-enhanced chest, abdomen, and pelvis CT. (10, 15) However, restaging for distant metastases after neoadjuvant RTX is not incorporated in these guidelines. (10, 15)

The local control, distant metastasis rates and progression-free survival between neoadjuvant and adjuvant RTX are comparable, indicating that delaying surgery because of neoadjuvant RTX does not influence oncological outcomes. Therefore, the standard of care of STS has evolved in most centres from surgical resection followed by RTX to RTX followed by surgery, taking the short- and long-term morbidity of adjuvant and neoadjuvant RTX into consideration. Surgery is usually planned 6-10 weeks after finishing neoadjuvant RTX. During this period previously undetectable or new metastases could develop, which could influence further management of the disease. Therefore, with the shift to neoadjuvant RTX the need to accurately assess distant disease is becoming increasingly important.

Restaging for distant metastases after neoadjuvant RTX has several advantages. In case of unresectable metastatic disease, resection of the primary tumour is likely not beneficial from an oncological point of view in most cases. (10) Through restaging, patients might therefore not undergo an often extensive operation. Nevertheless, in some cases resection of the primary tumour might still be beneficial and improve quality of life, for example in case of an ulcerating, bleeding, or painful tumour. Moreover, through restaging, some patients could benefit from metastasectomy of the timely detected metastases. (12, 23) Also, in case of indeterminate lesions, a restaging scan could help to differentiate between metastases and benign lesions. However, there are also some disadvantages of restaging such as the costs, radiation exposure, the prolonged uncertainty due to the finding of indeterminate lesions, and false-positive findings.

To our knowledge, this study is the first study to date that assessed the value of restaging for distant metastases in patients with STS of the extremities and trunk wall.

However, the value of restaging for distant metastases has been evaluated within other types of cancer, such as gastric cancer and locally advanced rectal cancer (LARC). (24-35) The results of restaging after neoadjuvant (chemo)radiotherapy in patients with LARC seem conflicting with a change in strategy rate varying from 0-15%. (26-35) A possible explanation of the conflicting results within these studies might be selection bias. It could be that restaging was not offered routinely in these studies and therefore only patients with a high likelihood of developing distant metastases or patients with complaints that could be caused by distant metastases received a restaging CT scan resulting in an overestimation of the value of routine restaging for distant metastases. Most of the studies did not report how many patients were restaged (26-30), however in some studies only 44-65% of the patients were restaged, suggesting some form of selection for restaging. (31-33) Contrary to these studies, the change in strategy rate in our centre for STS was 19% with a restaging rate of 93%, suggesting that selection bias in this study was limited. Nevertheless, in our centre neoadjuvant RTX was mainly indicated in patients with high-grade, large (>5cm), deep-seated tumours in which the members of the MDT meetings considered the estimated risk for local relapse as high. Therefore, restaging after neoadjuvant RTX might only be beneficial in these high-risk patients, who might also have a higher risk for the development of distant metastases.

A potential source of misclassification bias is the accuracy of chest CT and X-ray for the detection of distant metastases. Pulmonary nodules are frequently encountered on chest CT. However, there are no uniform definitions to distinguish from indeterminate and metastatic pulmonary lesions. Also, the Fleischner criteria for the evaluation of pulmonary nodules are not recommended for the use in patients with known primary cancers. (36) Furthermore, in literature a wide variety of definitions are used for indeterminate and metastatic pulmonary lesions. (37-40) In this study the investigators reviewed all radiology reports after an extensive literature search. Afterwards, the investigators designed a list of criteria to define indeterminate and metastatic lesions (**Appendix B**). This was reviewed by a dedicated radiologist. Based on these criteria all staging and restaging reports were reviewed and classified by the investigators. In our study only 2 out of 24 patients had a pathology confirmed metastasis. Both patients had a large solitary lesion suspected of distant metastasis. All other patients diagnosed with metastases at restaging had multiple lung lesions suspected of metastatic disease. In all these patients, follow-up CT scans showed (further) progression of the pulmonary nodes, which increases the likelihood of being truly metastatic lung lesions.

This study has some limitations due to its retrospective design. Besides the abovementioned limitations of our study, we were unable to find out why some patients

were not restaged with chest CT or X-ray (n=10). Therefore, selection bias could not be ruled out entirely. Furthermore, owing to the selected indication of neoadjuvant RTX, the findings of this study might not be generalizable to low-risk patients with small, superficial, low-grade tumours. Due to loss of follow-up, mainly because of referral of patients to secondary care in palliative setting, the received treatment after restaging was missing for some patients. Also, staging and restaging scans were not reassessed for this study by a blinded dedicated radiologist. Due to the relatively small numbers of patients, we were unable to assess risk factors that are associated with change of treatment strategy after restaging. Furthermore, we were unable to assess whether change of treatment strategy results in better quality of life compared to patients who did not receive restaging imaging for distant disease. However, this study is the first to date that shows that restaging for distant disease in STS results in a notable number of new findings which influences the clinical and patient's decision for further treatment and care. These findings should be further validated in prospective controlled studies to assess whether the change in treatment strategy due to findings on restaging improves quality adjusted life years. Furthermore, future studies are needed to assess which patients are most likely to benefit from restaging.

Conclusion

This study showed the value of routine restaging for distant metastases with chest CT or X-ray after neoadjuvant RTX in patients with STS of the trunk wall and extremities. Restaging imaging reveals a notable number of formerly unknown metastases and results in 19% of the patients in a change in treatment strategy.

References

1. Zagars GK, Ballo MT, Pisters PW, Pollock RE, Patel SR, Benjamin RS, et al. Prognostic factors for patients with localized soft-tissue sarcoma treated with conservation surgery and radiation therapy: an analysis of 1225 patients. Cancer. 2003;97(10):2530-43.
2. Acem I, Verhoef C, Rueten-Budde AJ, Grünhagen DJ, van Houdt WJ, van de Sande MAJ. Age-related differences of oncological outcomes in primary extremity soft tissue sarcoma: a multistate model including 6260 patients. European journal of cancer (Oxford, England : 1990). 2020;141:128-36.
3. Coindre JM, Terrier P, Guillou L, Le Doussal V, Collin F, Ranchère D, et al. Predictive value of grade for metastasis development in the main histologic types of adult soft tissue sarcomas: a study of 1240 patients from the French Federation of Cancer Centers Sarcoma Group. Cancer. 2001;91(10):1914-26.
4. Batista PB, Bertollo EMG, Costa DdS, Eliam L, Cunha KSG, Cunha-Melo JR, et al. Neurofibromatosis: part 2--clinical management. Arq Neuropsiquiatr. 2015;73(6):531-43.
5. Ferguson PC, Deheshi BM, Chung P, Catton CN, O'Sullivan B, Gupta A, et al. Soft tissue sarcoma presenting with metastatic disease: outcome with primary surgical resection. Cancer. 2011;117(2):372-9.
6. Bedi M, King DM, Charlson J, Whitfield R, Hackbarth DA, Zambrano EV, et al. Multimodality management of metastatic patients with soft tissue sarcomas may prolong survival. Am J Clin Oncol. 2014;37(3):272-7.
7. Lochner J, Menge F, Vassos N, Hohenberger P, Kasper B. Prognosis of Patients with Metastatic Soft Tissue Sarcoma: Advances in Recent Years. Oncol Res Treat. 2020;43(11):613-9.
8. Hong Z, England P, Rhea L, Hirbe A, McDonald D, Cipriano CA. Patterns of Extrapulmonary Metastases in Sarcoma Surveillance. Cancers (Basel). 2021;13(18).
9. Smolle MA, van Praag VM, Posch F, Bergovec M, Leitner L, Friesenbichler J, et al. Surgery for metachronous metastasis of soft tissue sarcoma - A magnitude of benefit analysis using propensity score methods. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2019;45(2):242-8.
10. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2021.
11. Chudgar NP, Brennan MF, Munhoz RR, Bucciarelli PR, Tan KS, D'Angelo SP, et al. Pulmonary metastasectomy with therapeutic intent for soft-tissue sarcoma. J Thorac Cardiovasc Surg. 2017;154(1):319-30.e1.
12. Blackmon SH, Shah N, Roth JA, Correa AM, Vaporciyan AA, Rice DC, et al. Resection of pulmonary and extrapulmonary sarcomatous metastases is associated with long-term survival. Ann Thorac Surg. 2009;88(3):877-84; discussion 84-5.
13. Canter RJ, Qin LX, Downey RJ, Brennan MF, Singer S, Maki RG. Perioperative chemotherapy in patients undergoing pulmonary resection for metastatic soft-tissue sarcoma of the extremity : a retrospective analysis. Cancer. 2007;110(9):2050-60.
14. Dossett LA, Toloza EM, Fontaine J, Robinson LA, Reed D, Druta M, et al. Outcomes and clinical predictors of improved survival in a patients undergoing pulmonary metastasectomy for sarcoma. Journal of surgical oncology. 2015;112(1):103-6.
15. Von Mehren M, Kane JM, Agulnik M, Bui MM, Ganjoo KN, George S, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. 2018;16(5):536-63.

16. Haas RL, Gronchi A, van de Sande MAJ, Baldini EH, Gelderblom H, Messiou C, et al. Perioperative Management of Extremity Soft Tissue Sarcomas. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(2):118-24.
17. Van Meekeren M, Fiocco M, Ho VKY, Bovée J, Gelderblom H, Haas RL. Patterns of Perioperative Treatment and Survival of Localized, Resected, Intermediate- or High-Grade Soft Tissue Sarcoma: A 2000-2017 Netherlands Cancer Registry Database Analysis. Sarcoma. 2021;2021:9976122.
18. O'Sullivan B, Davis AM, Turcotte R, Bell R, Catton C, Chabot P, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. Lancet. 2002;359(9325):2235-41.
19. O'Sullivan B, Davis A, Turcotte R, Bell R, Wunder J, Catton C, et al. Five-year results of a randomized phase III trial of pre-operative vs post-operative radiotherapy in extremity soft tissue sarcoma. Journal of Clinical Oncology. 2004;22(14_suppl):9007-.
20. Davis AM, O'Sullivan B, Turcotte R, Bell R, Catton C, Chabot P, et al. Late radiation morbidity following randomization to preoperative versus postoperative radiotherapy in extremity soft tissue sarcoma. Radiother Oncol. 2005;75(1):48-53.
21. WHO Classification of Tumours: Soft Tissue and Bone Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020.
22. R Development Core Team. R: A language and environment for statistical computing. 3.6.3 ed. Vienna, Austria: R Foundation for Statistical Computing; 2010.
23. Cariboni U, De Sanctis R, Giaretta M, Voulaz E, Morenghi E, Colombo P, et al. Survival Outcome and Prognostic Factors After Pulmonary Metastasectomy in Sarcoma Patients: A 18-Year Experience at a Single High-volume Referral Center. Am J Clin Oncol. 2019;42(1):6-11.
24. Gertsen EC, de Jongh C, Brenkman HJF, Mertens AC, Broeders I, Los M, et al. The additive value of restaging-CT during neoadjuvant chemotherapy for gastric cancer. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2020;46(7):1247-53.
25. Sandø AD, Fougner R, Grønbech JE, Bringeland EA. The value of restaging CT following neoadjuvant chemotherapy for resectable gastric cancer. A population-based study. World J Surg Oncol. 2021;19(1):212.
26. Ayez N, Alberda WJ, Burger JW, Eggermont AM, Nuyttens JJ, Dwarkasing RS, et al. Is restaging with chest and abdominal CT scan after neoadjuvant chemoradiotherapy for locally advanced rectal cancer necessary? Annals of surgical oncology. 2013;20(1):155-60.
27. Bisschop C, Tjalma JJ, Hospers GA, Van Geldere D, de Groot JW, Wiegman EM, et al. Consequence of restaging after neoadjuvant treatment for locally advanced rectal cancer. Annals of surgical oncology. 2015;22(2):552-6.
28. McBrearty A, McCallion K, Moorehead RJ, McAllister I, Mulholland K, Gilliland R, et al. Re-Staging Following Long-Course Chemoradiotherapy For Rectal Cancer: Does It Influence Management? Ulster Med J. 2016;85(3):178-81.
29. Hanly AM, Ryan EM, Rogers AC, McNamara DA, Madoff RD, Winter DC. Multicenter Evaluation of Rectal cancer ReImaging pOst Neoadjuvant (MERRION) Therapy. Ann Surg. 2014;259(4):723-7.
30. Schneider DA, Akhurst TJ, Ngan SY, Warrier SK, Michael M, Lynch AC, et al. Relative Value of Restaging MRI, CT, and FDG-PET Scan After Preoperative Chemoradiation for Rectal Cancer. Dis Colon Rectum. 2016;59(3):179-86.
31. Jaffe TA, Neville AM, Bashir MR, Uronis HE, Thacker JM. Is follow-up CT imaging of the chest and abdomen necessary after preoperative neoadjuvant therapy in rectal cancer patients without evidence of metastatic disease at diagnosis? Colorectal Dis. 2013;15(11):e654-8.

32. Caturegli I, Molin MD, Laird C, Molitoris JK, Bafford AC. Limited Role for Routine Restaging After Neoadjuvant Therapy in Locally Advanced Rectal Cancer. The Journal of surgical research. 2020;256:317-27.
33. Park HJ, Jang JK, Park SH, Park IJ, Kim JH, Baek S, et al. Restaging Abdominopelvic Computed Tomography Before Surgery After Preoperative Chemoradiotherapy in Patients With Locally Advanced Rectal Cancer. JAMA Oncol. 2018;4(2):259-62.
34. Davids JS, Alavi K, Andres Cervera-Servin J, Choi CS, Sturrock PR, Sweeney WB, et al. Routine preoperative restaging CTs after neoadjuvant chemoradiation for locally advanced rectal cancer are low yield: a retrospective case study. Int J Surg. 2014;12(12):1295-9.
35. Liu GC, Zhang X, Xie E, An X, Cai PQ, Zhu Y, et al. The Value of Restaging With Chest and Abdominal CT/MRI Scan After Neoadjuvant Chemoradiotherapy for Locally Advanced Rectal Cancer. Medicine (Baltimore). 2015;94(47):e2074.
36. MacMahon H, Naidich DP, Goo JM, Lee KS, Leung ANC, Mayo JR, et al. Guidelines for Management of Incidental Pulmonary Nodules Detected on CT Images: From the Fleischner Society 2017. Radiology. 2017;284(1):228-43.
37. Saifuddin A, Baig MS, Dalal P, Strauss SJ. The diagnosis of pulmonary metastases on chest computed tomography in primary bone sarcoma and musculoskeletal soft tissue sarcoma. Br J Radiol. 2021;94(1123):20210088.
38. Saifuddin A, Shafiq H, Rajakulasingam R, Tan A, O'Donnell P, Khoo M. A review of staging chest CT in trunk and extremity soft tissue sarcoma. Br J Radiol. 2021;94(1118):20201109.
39. Mayo Z, Kennedy S, Gao Y, Miller BJ. What Is the Clinical Importance of Incidental Findings on Staging CT Scans in Patients With Sarcoma? Clin Orthop Relat Res. 2019;477(4):730-7.
40. Mao Y, Hedgire S, Harisinghani M. Radiologic Assessment of Lymph Nodes in Oncologic Patients. Current Radiology Reports. 2013;2(2):36.

Appendix

Appendix A. Variable definitions

Age was determined as age at start of neoadjuvant RTX. Size was measured as the maximum diameter of tumour mass on imaging-techniques or based on pathological report. The Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system was used for tumour grading. A tumour partially or entirely deep to the investing fascia was classified as deep. Histological subtypes were retrieved from pathology reports and were classified into 7 categories according to the World Health Organization classification (1): leiomyosarcoma (LMS), liposarcoma (LPS), myxofibrosarcoma (MF), undifferentiated pleomorphic sarcoma and (pleomorphic) STS not-otherwise-specified (UPS/NOS), malignant peripheral nerve sheath tumour (MPNST), synovial sarcoma (SS) and other. The 'other'-category included angiosarcoma, alveolar rhabdomyosarcoma and other histological subtypes underrepresented in our data. Surgical margin was classified according to the R-classification with R0 (negative, defined as no ink on tumour), R1 (microscopically positive), and R2 (macroscopically positive). Findings at staging and restaging chest CT scan/X-ray were categorized as not suspected, indeterminate, or metastases.

Appendix B. The classification of indeterminate and metastatic lesions

Although previous studies have attempted to determine CT/X-ray features that allow for reliable diagnosis of pulmonary metastases (2), there is no consensus definitions for metastatic and indeterminate nodules based on chest CT/X-ray. Nodules were considered metastases if they were confirmed histologically or if the appearance of the nodules was highly suspicious for metastatic disease.

Lung nodules were considered metastatic if:

- multiple non-calcified nodules were present with at least one nodule ≥10 mm, or
- pre-existing nodules showed an obvious progression in number and or in size, or
- a new non-calcified nodule developed of ≥10 mm, or
- multiple new non-calcified nodules developed.

Lymph nodes were considered metastatic if:

- evident visible progression of suspected irregular lymph nodes was present, or
- new irregular lymph nodes with a short-axis diameter ≥10 mm developed. (3)

Nodules of other locations were considered metastatic if:

- pre-existing nodules showed an obvious progression in number and or in size, or
- a new node developed of ≥20 mm, and

- the appearance was not clearly suggestive of a specific benign process such as granuloma, cyst, or haemangioma

Indeterminate lesions were nodules of which the radiologists could not distinguish whether the lesion was benign or malignant.

Lesions were classified as indeterminate if:

- the appearance was not clearly suggestive of a specific benign process such as granuloma, cyst, or haemangioma and could be representative of an early metastasis (4), and
- multiple lung nodules of <5 mm or a solidary lung nodule of 5-10 mm were present, or
- Additional diagnostic tests or imaging were performed to determine the nature of the nodule or if the radiologist advised surveillance of the lesions.

Lesions that were tumour-positive after additional diagnostic tests were classified as metastatic disease. Solitary lung lesions of <5 mm were usually considered not suspected. **Figure 1** depicts the algorithm used in this study for the classification of lung lesions.

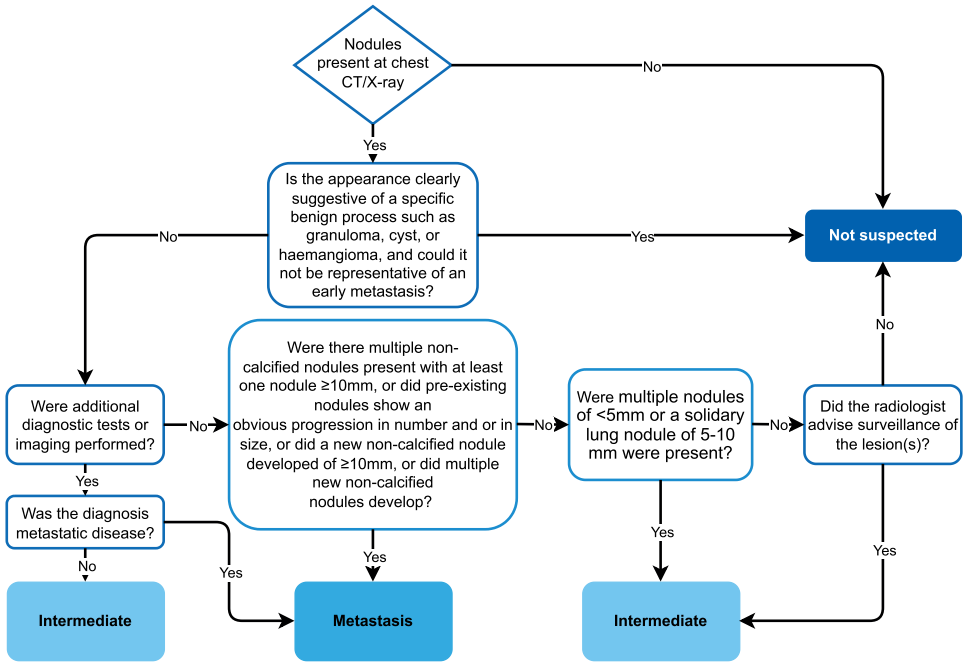

Figure 1. Work-up for the classification of lung lesion

References

1. WHO Classification of Tumours: Soft Tissue and Bone Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020.
2. Saifuddin A, Baig MS, Dalal P, Strauss SJ. The diagnosis of pulmonary metastases on chest computed tomography in primary bone sarcoma and musculoskeletal soft tissue sarcoma. Br J Radiol. 2021;94(1123):20210088.
3. Mao Y, Hedgire S, Harisinghani M. Radiologic Assessment of Lymph Nodes in Oncologic Patients. Current Radiology Reports. 2013;2(2):36.
4. Mayo Z, Kennedy S, Gao Y, Miller BJ. What Is the Clinical Importance of Incidental Findings on Staging CT Scans in Patients With Sarcoma? Clin Orthop Relat Res. 2019;477(4):730-7.

Chapter 11

Prediction Tools for Personalised Management of Soft Tissue Sarcoma of the Extremity

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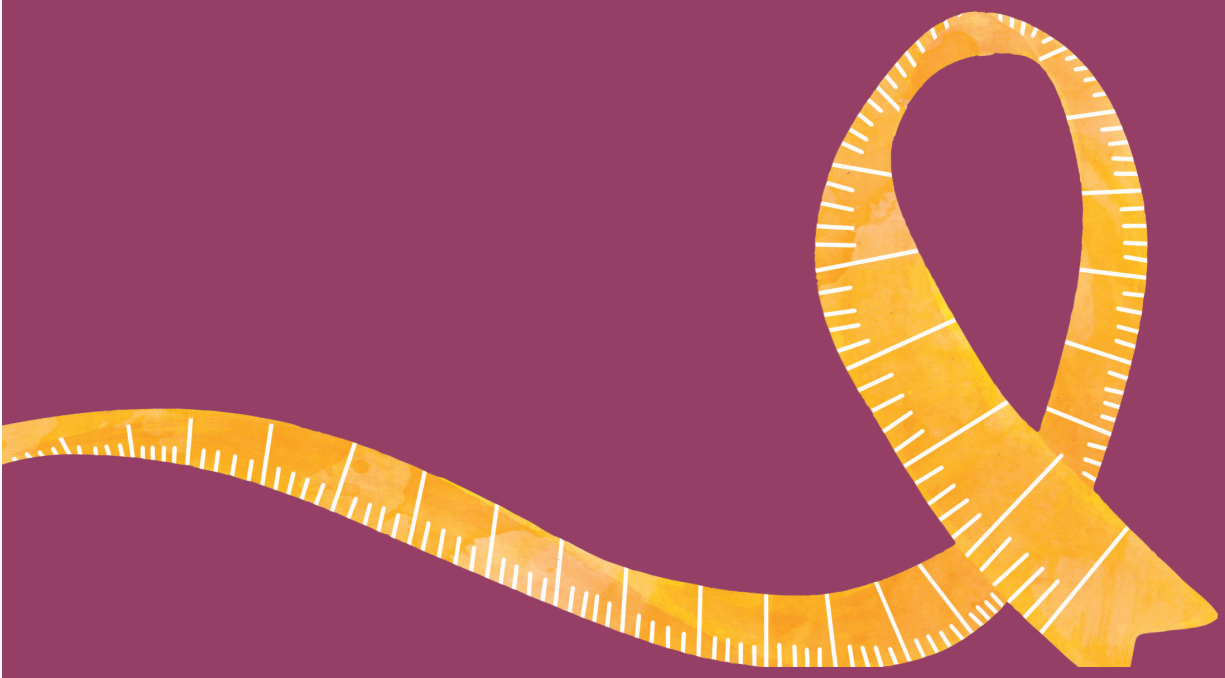

Abstract

Prediction tools are instruments which are commonly used to estimate the prognosis in oncology and facilitate clinical decision-making in a more personalized manner. Their popularity is shown by the increasing numbers of prediction tools, which have been described in the medical literature. Many of these tools have been shown to be useful in the field of soft-tissue sarcoma of the extremities (eSTS). In this annotation, we aim to provide an overview of the available prediction tools for eSTS, provide an approach for clinicians to evaluate the performance and usefulness of the available tools for their own patients, and discuss their possible applications in the management of patients with an eSTS.

Introduction

Soft tissue sarcomas (STS) represent a group of rare and heterogeneous malignant neoplasms encompassing over 100 different histological subtypes. (1) STSs arise from mesenchymal cells and account for 1% of all adult malignancies. (2) The estimated incidence is 4.71 per 100,000 people per year in Europe. (3) STS may occur in any anatomic site, but the extremities are the most common primary site for STS. (4, 5) Because of the heterogeneity in presentation and outcome within the spectrum of extremity soft tissue sarcoma (eSTS), several prognostic instruments have been developed to classify patients with eSTS in several risk groups to optimize the management of eSTS. Historically, conventional staging systems such as the American Joint Committee on Cancer (AJCC) TNM classification were widely used for stratification of patients. (6) However, important prognostic patient and tumour-related factors such as age and histological subtype are not incorporated in the TNM staging system. In recent years, several new prognostic instruments such as prediction tools and nomograms have been developed for eSTS. In general, these tools are easier to use though applications on smartphones, more accurate as they generate individual prognosis based on multiple characteristics that may vary simultaneously, and provide more easily understood prognosis compared with conventional staging systems. In this annotation, we will discuss the current concepts of managing eSTS, explore the available prediction tools for eSTS, provide clinicians and researchers instruments to assess which prediction tool to use, and discuss current and future applications of prediction tools for clinical decision making and personalized management in eSTS.

Management of eSTS

Several clinical guidelines have been developed for the management of eSTS. (7, 8) The treatment of eSTS should occur in a multidisciplinary team using a multimodality approach. Several studies have demonstrated that treatment for STS in high-volume centres is associated with better oncological outcomes. (5, 9-11) This underlines the importance of centralization of sarcoma care in centres with a dedicated sarcoma team.

Surgery and radiotherapy

Surgery with complete surgical margins is the standard treatment in localized eSTS. (Neo)adjuvant radiotherapy (RTX) is typically indicated in high-grade eSTS with a high risk of local relapse or incomplete surgical margins. The most important factors influencing RTX recommendation are (anticipated) surgical margin, tumour grade, histological subtype, tumour size and location. (12) It has been shown that a marginal resection after RTX may not compromise local control or overall survival. (13, 14)

Also, recent studies suggest that after a R1 excision (microscopic residual disease) or unplanned excision, re-excision may be postponed after multidisciplinary discussion until a local relapse occurs, without compromising overall survival or distant control. (15, 16) However, the clinical guidelines recommend systematic re-excision in case of incomplete surgical margins if R0 re-resection is feasible. (7, 8)

Timing of radiotherapy

There is no clear preference concerning the timing of RTX. Local control and overall survival are comparable after neoadjuvant and adjuvant RTX. (17-20) Traditionally, RTX was often offered postoperatively, as short term wound complications are less common after adjuvant RTX. However, neoadjuvant RTX results in less long-term morbidity such as fibrosis, oedema and joint stiffness compared with adjuvant RTX. (17-20) Given that the short term-complications are well manageable in specialized sarcoma centres, RTX is nowadays typically offered preoperatively. (7, 21)

Chemotherapy

(Neo)adjuvant chemotherapy (CTX) may be indicated in patients with a high risk of developing distant metastasis (DM) or death. Perioperative CTX is not standard treatment in the management of primary eSTS but could be offered in a selected group of high-risk patients after multidisciplinary discussion. Chemosensitivity of the histological subtype should be taken into consideration.

Despite multiple randomized and non-randomized studies on the added value of perioperative CTX in eSTS, the role of CTX is still widely debated. (22-34) To date, five randomized trials comparing anthracycline and ifosfamide-based (neo) adjuvant CTX in addition to standard treatment vs standard treatment alone have been performed. (22-26) None of these studies found a survival benefit in the CTX arm in the total study population. However, most trials included patients with low grade tumours and small superficial tumours, which are considered low risk patients. In addition, three of the five trials were closed prematurely because of poor patient accrual. (23, 25, 26)

Recent studies demonstrated a survival benefit for anthracycline and ifosfamide based CTX in localized eSTS in a selected group of high-risk patients. (33-35) These high-risk patients were identified by prediction tools. These tools predict individual patient risks of death and DM based on patient, tumour and treatment characteristics. (36, 37) A survey among sarcoma specialists showed that 81% of the specialists consider the use of a prediction tool for the indication of (neo)adjuvant chemotherapy in primary eSTS. (12) This marks the recent trend of a more patient-tailored approach in the management of eSTS.

Treatment with (neo)adjuvant isolated limb perfusion with tumour necrosis factor-alpha plus melphalan and (neo)adjuvant regional hyperthermia combined with CTX may also be an option for limb-preserving treatment after multidisciplinary discussion in reference centres. (7, 8, 38)

Development of prediction tools for personalized prognosis

Prediction tools in the form of a nomogram or a computer or smartphone-based calculator are commonly used to estimate oncological events such as the risk of relapse and death in medicine. These tools generate individual probabilities of an event based on a combination of factors accounting for the fact that patients have multiple characteristics that vary simultaneously. This results in a more accurate individual prognosis which is easier to explain compared with conventional staging systems in cancer. Prediction tools facilitate the clinical decision-making process towards a more patient-tailored manner. The last decade there has been an enormous increase in the development and publication of prognostic tools in medicine. Also, in the field of eSTS several prognostic tools have been developed. (36, 37, 39-47)

An overview of published prediction tools for patients with primary STS are included in **Table 1**. Diagnostic models and histology specific models are not included in this overview. All prediction tools differ in the inclusion criteria. Three tools included only STS of the extremities while other studies included sarcomas of other sites. (36, 37, 42) Some studies included patients with metastatic disease or local recurrence at presentation. (41, 44-47) One study combined bone and soft tissue tumours in the prediction tool. (46) All prediction tools included sarcoma-specific survival (SSS) or overall survival (OS) as an outcome of the model (36, 37, 39, 41, 44-47), except from the nomogram of Cahlon et al. (42) Only four studies were externally validated. (36, 37, 39, 41)

Two prediction tools, Sarculator and PERSARC, included dynamic predictions. (48, 49) Both dynamic tools were externally validated. (49, 50) Prediction tools usually predict oncological outcomes at a certain timepoint (e.g. 5-year OS) at time of surgery. However, the prognosis of a patient may change as time proceeds. For example, the longer the patients being disease free after surgery, the lower the chance of disease recurrence and the better the prognosis, and patients who eventually develop disease recurrence during follow-up will have a worse prognosis compared to patients who remain disease free after surgery. Dynamic predictions take these time-varying variables into account, and can predict the prognosis at various time points during follow-up.

All prediction tools in eSTS include patient and tumour specific characteristics. Five out of nine studies included also treatment related covariates in their nomogram. (36, 42, 44, 46, 47) Besides these clinical predictors, the prognostic ability of various other factors such as gene expression profiles, radiomics, transcriptomics, proteomics and other (multi-)omics are widely investigated. (51-57) However, the assessment of the added value of these promising predictors and models, compared with the existing clinical prediction tools, and further external validation are warranted.

Table 1. Overview of published prognostic tools for soft tissue sarcoma (excl. histology specific tools)

Study (name)	Population	Primary endpoint	Predictors	Dynamic predictions	Validation
Kattan et al., 2002, Mariani et al., 2005 (MSKSN) (39, 40)	Patients (>16 years of age) with primary, non-metastatic, STS treaded with surgery	12-year SSS	Age, size (cat), grade, histological subtype, depth, site	No	External (58, 59)
Sampo et al., 2012 (41)	Patients (>16 years of age) with non-metastatic primary or locally recurrent eSTS or trunk wall STS	10-year SSS	Size (cat), grade, depth, site, necrosis, vascular invasion	No	External (41)
Cahlon et al., 2012 (42)	Patients (>16 years of age) with primary, non-metastatic, eSTS treated with limb sparing surgery alone (excluding perioperative RTX and CTX)	3- and 5-year LR	Age (cat), size (cat), grade, histological subtype, margin	No	Internal (42)
Callegaro et al., 2016 (Sarculator) (37)	Patients (>18 years of age) with primary (non-recurrent and non-metastatic) eSTS operated with curative intent	5- and 10-year OS, 5- and 10-year DM	Age, size, grade, histological subtype	Yes (49)	External (37, 49, 59, 60)
Van Praag et al., 2017, Smolle et al., 2019 (PERSARC) (36, 43)	Patients (>18 years of age) with high-grade, primary (non-recurrent and non-metastatic) eSTS operated with curative intent	3-, 5- and 10-year OS, 3-, 5- and 10-year DM, 3-, 5- and 10-year LR	Age, size, grade, histological subtype, depth, margin, RTX	Yes (48)	External (43, 50)
Sekimizu et al., 2019 (44)	Patients (>18 years of age) with primary (N0M0 or N1M0), eSTS and trunk STS operated with curative intent	2-year LR, 2-year DM, and 2-year OS	Age (cat), size, grade, histological subtype, depth, site, margin, sex, nodal metastasis	No	Internal (44)
Zhang et al., 2019 (45)	Patients (>18 years of age) with primary STS surgically treated	3- and 5-year OS, 3- and 5-year SSS	Age (cat), size (cat), grade, histological subtype, sex, stage [a], marital status, insurance status	No	Internal (45) [b]
Xu et al., 2020 (46)	Patients with bone and soft tissue tumours (except from the heart)	3-month OS, 3-month SSS, 3-month non-SSS	Age (cat), grade, site, surgery, sex, stage [a], T-stage, brain metastasis, lung metastasis, laterality, race	No	No
Tu et al., 2021 (47)	Patients with primary STS	1-, 2-, and 3-year OS	Age (cat), size (cat), grade, histological subtype, surgery, RTX, CTX, lung metastasis	No	Internal (47) [b]

STS: soft tissue sarcoma, eSTS: soft tissue sarcoma of the extremity, cat: categorical, SSS: sarcoma-specific survival, OS: overall survival, DM: distant metastasis rate, LR: local recurrence rate, RTX: radiotherapy, CTX: chemotherapy

[a] Stage includes localized, regional or distant disease

[b] In the paper stated as external validation, however the validation cohort was a random split from the same source population (training and validation cohort both from SEER dataset), which is considered internal validation. (61)

Model performance

After a careful model building process, an assessment of how good the predictions of a model are, need to take place. Model performance is often expressed in discrimination and calibration.

Discrimination

Discrimination relates to how well the model could distinguish between who experienced an event and who did not. Discrimination is measured by the area under the curve (AUC) of a received operating curve (ROC), also known as the concordance index, Harrell's c-index or c-index. The ROC curve is a plot of the sensitivity (true positive rate) against the 1 – specificity (false-positive rate) for different cut-off values of the probability of an outcome. The Harrell's c-index for survival models is the probability that for all possible patient pairs, the patient with a shorter time-to-event has a higher predicted risk of the event compared to the paired patient with a longer time-to-event. A c-index of 0.5 corresponds to a model that is no better than chance and a c-index of 1 corresponds to perfect discrimination (the model could perfectly distinguish patients with a shorter time-to-event from the patient with a longer time-to-event).

Calibration

Calibration estimates how close the predicted risk based on the prediction tool is to the observed risk in the study population. Calibration could be assessed visually in a calibration plot in which the observed probability is plotted against the predicted probability. The 45 degrees line in a calibration plot indicates perfect calibration (predicted and observed probability are equal). For survival data, the calibration plot is often reported for several clinically relevant time points.

Both discrimination and calibration are not intrinsic properties of a model. These measurements evaluate how well the model performs in a particular cohort. A good discriminative ability is important for risk-stratification and to identify a high-risk subgroup, while a good calibration is important for informing patients about their prognosis and clinical decision making.

Internal vs. external validation

The best assessment of model performance is in an external validation cohort. Validation is the process of assessing the model performance on different populations and the applicability (generalizability) to these populations. Most prediction tools in eSTS were only validated internally. (42, 44, 45, 47) Internal validation assesses validity for the setting where the training or development data originated from. It assesses the

reproducibility of the model in the same underlying population. External validation assesses the validity in a fully independent cohort. The book '*Clinical Prediction Models*' of E.W. Steyerberg provides a practical approach for and further explanation of different techniques of internal and external validation. (61) Poor external validation of a model may often be explained by inadequate model development, overfitting due to a relatively small sample size with many candidate predictors or a single centre development cohort.

Model update

Poor external validation may also be related to true differences between the development and validation cohort. Prediction tools should be updated for new settings (e.g., difference in time). This could be done by re-calibration, re-estimation of regression coefficients or model extension with new predictors. For example, one may argue that the accuracy of the predictions of a generic eSTS model in a patient with a malignant peripheral nerve sheath tumour (MPNST) of the extremity, would be less than based on a MPNST specific prediction tool in which important MPNST-specific predictors, e.g., the presence of neurofibromatosis type 1 and rhabdomyoblastic differentiation (triton tumour), are incorporated. (62) A recent study showed that the discriminative ability of the Sarculator is less in MPNSTs compared with other histological subtypes, such as leiomyosarcomas (C-index: 0.66 vs 0.75, respectively). (60) This could be a reason to update the Sarculator in the MPNST setting with additional important MPNST-specific predictors. For the extension of prediction tools, a trade-off between predictive value and usability or availability to assess the new predictor in clinical practice, should be made. Several approaches for updating existing prediction models are described by E.W. Steyerberg. (61)

Use of prediction tool for personalized care

Formerly, patients with a larger than 5 cm, deep-seated, high-grade tumour, were considered 'high risk' patients. (63) However, the updated ESMO guideline of 2021 no longer uses this definition for high-risk patients and states that prognostic tools, such as Sarculator and PERSARC, could be used to identify high risk patients for e.g., the indication of (neo)adjuvant CTX. (7) Both prognostic tools are available as application that could be downloaded in the Apple App Store and Google Play Store.

Choice for prediction tool

Given the variety in eligibility criteria and the differences in the development and validation populations, it is difficult to compare the performance of the prediction tools based on their reported discriminative ability and other model performance measures. For the choice which prediction tool to use in clinical setting, one should

assess whether the source population (development cohort) or the external validation cohort of the nomogram is comparable with its own patient population. Furthermore, the outcome of interest and relevance and availability of the used prognostic covariates in the model should guide the choice for prediction tool.

Decision curve analysis

Besides the applicability of the prediction tool in the physician's own patient population and the corresponding performance outcomes, the clinical usefulness should be assessed. This could be done with a decision curve analysis (DCA). In a DCA the net benefit of a prediction tool-assisted decision at different threshold probabilities is depicted and compared with the default decision of an intervention for all patients and an intervention for none of the patients. The net benefit is defined as the fraction of true positives subtracted by the fraction of false positives at a certain threshold probability, weighted by the relative harm of a false positive and a false negative result. (64) This weight corresponds to the harm (false positive) to benefit (false negative) - ratio. (65) For example, if we would accept 4 false positives for one true positive, this would correspond to a threshold probability of 20% and a harm to benefit-ratio of 4, which means that missing a true positive is 4 times worse than having a false positive.

PERSARC for CTX indication

Figure 1 depicts the DCA of the PERSARC prediction tool in a multicentre cohort of patients with a high-grade eSTS as described by I. Acem et al. (33) As previously described, a majority of sarcoma specialists would consider the use of a prediction tool for the indication of (neo)adjuvant CTX. (12) This DCA illustrates that the PERSARC tool will be clinically useful for the indication of (neo)adjuvant CTX if physicians treat patients with eSTS with a predicted 5-year mortality between 6% and 45%. The threshold probability refers to the preference of a physician and reflects how physicians value different outcomes for their patients. If a physician is willing to offer (neo)adjuvant CTX for patients with a predicted 5-year mortality of less than 6% (5-year survival of more than 94%), the physician should treat all patients with (neo) adjuvant CTX and the prediction tool will not be clinically informative. If a physician is willing to treat patients only if they have a predicted 5-year mortality of more than 45% (5-year survival of less than 55%), the physician should treat none of the patients with (neo)adjuvant chemotherapy. Again, in this situation the prediction tool will not be clinically informative. If the threshold probability of a physician lies within the range of 6% and 45%, taking the relative harm and benefit of (avoiding) treatment with (neo)adjuvant CTX into account, the PERSARC model is clinically useful. In the study of I. Acem et al. (33), the authors found a survival benefit for (neo)adjuvant anthracycline and ifosfamide-based CTX in a subgroup of patients with a 5-year

predicted OS of ≤66% (5-year predicted mortality of 34%). This lies within the range of threshold probabilities in which the model is clinically useful.

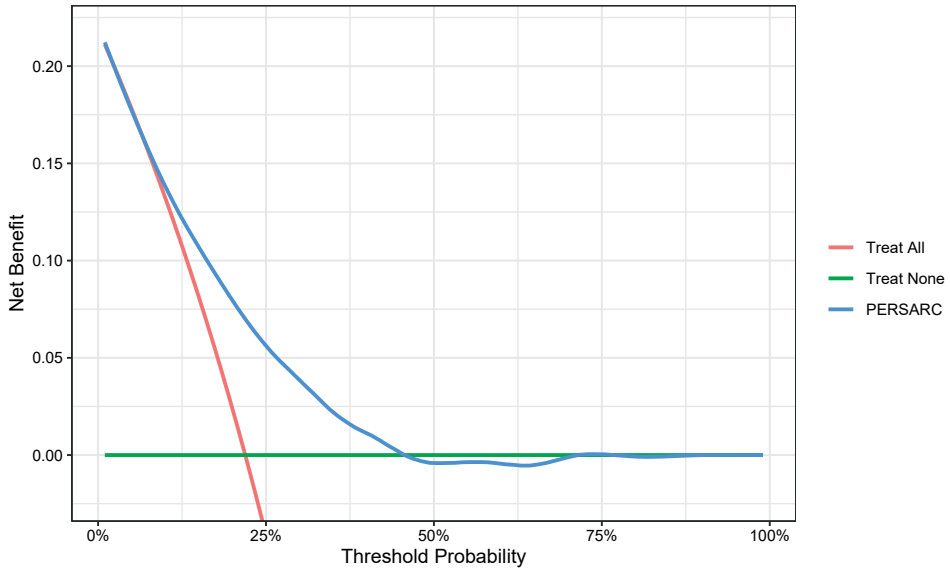

Figure 1. Clinical decision curve using the PERSARC prediction tool

Clinical applications

Besides the use of prediction tools for the indication of (neo)adjuvant treatment, (33, 34) prediction tools provide an opportunity to tailor counselling and follow-up schedules.

Prediction tools could help physicians to inform their patients about their prognosis and to guide clinical decision making. However, there is limited knowledge on patient comprehension, satisfaction and quality of life (QoL) with the use of prediction tools in the management of eSTS. Therefore, the PERSARC research group has started a randomized trial (www.trialregister.nl/trial/9160) to assess whether the use of PERSARC as decision supporting intervention could contribute to a better-informed choice, less decisional conflict and improved QoL from patients' perspective.

Furthermore, dynamic prognostic tools could be useful for tailoring follow-up regimens to the risk of tumour recurrence. The PERSARC group recently published a study in which conditional risks for LR and DM were predicted using flexible parametric competing risk regression models. (43) However, the optimal risk threshold upon which an individual patient needs to visit the outpatient clinic or undergo imaging,

should be further evaluated though for example (microsimulation) decision modelling for cost-effectiveness. (66)

Finally, prediction tools are very useful in research, for instance, for risk-stratified analysis to assess treatment heterogeneity in clinical trials, (67) and for selection of patients for randomized clinical trials. (68)

Conclusion and future perspectives

Prediction tools are important instruments for clinical decision making in the modern world and facilitate the shift from a one-size-fits-all approach to patient-tailored management of eSTS. Prediction tools demonstrated to be valuable for the identification of high-risk patients that benefit from (neo)adjuvant anthracycline and ifosfamide-based CTX. (33, 34) Further development of existing tools with other promising predictors and re-calibration and re-estimation for different settings are warranted for a good application of the tools in clinical practice. For the extension of prediction tools, a trade-off between predictive value and ability to obtain the predictor in clinical practice should be made, balancing precision and usability.

References

1. WHO Classification of Tumours: Soft Tissue and Bone Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA: a cancer journal for clinicians. 2021;71(1):7-33.
3. Gatta G, Capocaccia R, Botta L, Mallone S, De Angelis R, Ardanaz E, et al. Burden and centralised treatment in Europe of rare tumours: results of RARECAREnet-a population-based study. The Lancet Oncology. 2017;18(8):1022-39.
4. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. European journal of cancer (Oxford, England : 1990). 2013;49(3):684-95.
5. Blay JY, Honoré C, Stoeckle E, Meeus P, Jafari M, Gouin F, et al. Surgery in reference centers improves survival of sarcoma patients: a nationwide study. Annals of oncology : official journal of the European Society for Medical Oncology. 2019;30(7):1143-53.
6. Edge SBESBAJCoC. AJCC cancer staging manual 8th ed. New York: Springer.; 2017.
7. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2021;32(11):1348-65.
8. Von Mehren M, Kane JM, Agulnik M, Bui MM, Ganjoo KN, George S, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. 2018;16(5):536-63.
9. Vos M, Blaauwgeers HGT, Ho VKY, van Houdt WJ, van der Hage JA, Been LB, et al. Increased survival of non low-grade and deep-seated soft tissue sarcoma after surgical management in high-volume hospitals: a nationwide study from the Netherlands. European journal of cancer (Oxford, England : 1990). 2019;110:98-106.
10. Lazarides AL, Kerr DL, Nussbaum DP, Kreulen RT, Somarelli JA, Blazer DG, 3rd, et al. Soft Tissue Sarcoma of the Extremities: What Is the Value of Treating at High-volume Centers? Clin Orthop Relat Res. 2019;477(4):718-27.
11. Venigalla S, Nead KT, Sebro R, Guttmann DM, Sharma S, Simone CB, 2nd, et al. Association Between Treatment at High-Volume Facilities and Improved Overall Survival in Soft Tissue Sarcomas. Int J Radiat Oncol Biol Phys. 2018;100(4):1004-15.
12. Acem I, Smit MM, Verhoef C, van Houdt WJ, Haas RL, van der Hage JA, et al. Management of Soft Tissue Sarcomas in Extremities: Variation in Treatment Recommendations and Surveillance According to Specialty and Continent. Annals of surgical oncology. 2021;28(12):7923-36.
13. Dagan R, Indelicato DJ, McGee L, Morris CG, Kirwan JM, Knapik J, et al. The significance of a marginal excision after preoperative radiation therapy for soft tissue sarcoma of the extremity. Cancer. 2012;118(12):3199-207.
14. Gundle KR, Kafchinski L, Gupta S, Griffin AM, Dickson BC, Chung PW, et al. Analysis of Margin Classification Systems for Assessing the Risk of Local Recurrence After Soft Tissue Sarcoma Resection. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(7):704-9.
15. Danieli M, Barretta F, Fiore M, Radaelli S, Sangalli C, Barisella M, et al. Unplanned Excision of Extremity and Trunk Wall Soft Tissue Sarcoma: To Re-resect or Not to Re-resect? Annals of surgical oncology. 2021;28(8):4706-17.
16. Decanter G, Stoeckle E, Honore C, Meeus P, Mattei JC, Dubray-Longeras P, et al. Watch and Wait Approach for Re-excision After Unplanned Yet Macroscopically Complete Excision of

Extremity and Superficial Truncal Soft Tissue Sarcoma is Safe and Does Not Affect Metastatic Risk or Amputation Rate. Annals of surgical oncology. 2019;26(11):3526-34.
17. Haas RL, Gronchi A, van de Sande MAJ, Baldini EH, Gelderblom H, Messiou C, et al. Perioperative Management of Extremity Soft Tissue Sarcomas. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(2):118-24.
18. O'Sullivan B, Davis AM, Turcotte R, Bell R, Catton C, Chabot P, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. Lancet. 2002;359(9325):2235-41.
19. O'Sullivan B, Davis A, Turcotte R, Bell R, Wunder J, Catton C, et al. Five-year results of a randomized phase III trial of pre-operative vs post-operative radiotherapy in extremity soft tissue sarcoma. Journal of Clinical Oncology. 2004;22(14_suppl):9007-.
20. Davis AM, O'Sullivan B, Turcotte R, Bell R, Catton C, Chabot P, et al. Late radiation morbidity following randomization to preoperative versus postoperative radiotherapy in extremity soft tissue sarcoma. Radiother Oncol. 2005;75(1):48-53.
21. Van Meekeren M, Fiocco M, Ho VKY, Bovée J, Gelderblom H, Haas RL. Patterns of Perioperative Treatment and Survival of Localized, Resected, Intermediate- or High-Grade Soft Tissue Sarcoma: A 2000-2017 Netherlands Cancer Registry Database Analysis. Sarcoma. 2021;2021:9976122.
22. Woll PJ, Reichardt P, Le Cesne A, Bonvalot S, Azzarelli A, Hoekstra HJ, et al. Adjuvant chemotherapy with doxorubicin, ifosfamide, and lenograstim for resected soft-tissue sarcoma (EORTC 62931): a multicentre randomised controlled trial. The Lancet Oncology. 2012;13(10):1045-54.
23. Fakhrai N, Ebm C, Kostler WJ, Jantsch M, Abdolvahab F, Dominkus M, et al. Intensified adjuvant IFADIC chemotherapy in combination with radiotherapy versus radiotherapy alone for soft tissue sarcoma: long-term follow-up of a prospective randomized feasibility trial. Wiener klinische Wochenschrift. 2010;122(21-22):614-9.
24. Frustaci S, Gherlinzoni F, De Paoli A, Bonetti M, Azzarelli A, Comandone A, et al. Adjuvant chemotherapy for adult soft tissue sarcomas of the extremities and girdles: results of the Italian randomized cooperative trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(5):1238-47.
25. Gortzak E, Azzarelli A, Buesa J, Bramwell VH, van Coevorden F, van Geel AN, et al. A randomised phase II study on neo-adjuvant chemotherapy for 'high-risk' adult soft-tissue sarcoma. European journal of cancer (Oxford, England : 1990). 2001;37(9):1096-103.
26. Petrioli R, Coratti A, Correale P, D'Aniello C, Grimaldi L, Tanzini G, et al. Adjuvant epirubicin with or without Ifosfamide for adult soft-tissue sarcoma. Am J Clin Oncol. 2002;25(5):468-73.
27. Zaidi MY, Ethun CG, Tran TB, Poultsides G, Grignol VP, Howard JH, et al. Assessing the Role of Neoadjuvant Chemotherapy in Primary High-Risk Truncal/Extremity Soft Tissue Sarcomas: An Analysis of the Multi-institutional U.S. Sarcoma Collaborative. Annals of surgical oncology. 2019;26(11):3542-9.
28. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Impact of perioperative chemotherapy and radiotherapy in patients with primary extremity soft tissue sarcoma: retrospective analysis across major histological subtypes and major reference centres. European journal of cancer (Oxford, England : 1990). 2018;105:19-27.
29. Mullen JT, Kobayashi W, Wang JJ, Harmon DC, Choy E, Hornicek FJ, et al. Long-term follow-up of patients treated with neoadjuvant chemotherapy and radiotherapy for large, extremity soft tissue sarcomas. Cancer. 2012;118(15):3758-65.
30. Pervaiz N, Colterjohn N, Farrokhyar F, Tozer R, Figueredo A, Ghert M. A systematic meta-analysis of randomized controlled trials of adjuvant chemotherapy for localized resectable soft-tissue sarcoma. Cancer. 2008;113(3):573-81.

31. Istl AC, Ruck JM, Morris CD, Levin AS, Meyer CF, Johnston FM. Call for improved design and reporting in soft tissue sarcoma studies: A systematic review and meta-analysis of chemotherapy and survival outcomes in resectable STS. Journal of surgical oncology. 2019;119(7):824-35.
32. Graham DS, van Dams R, Jackson NJ, Onyshchenko M, Eckardt MA, DiPardo BJ, et al. Chemotherapy and Survival in Patients with Primary High-Grade Extremity and Trunk Soft Tissue Sarcoma. Cancers (Basel). 2020;12(9).
33. Acem I, van Houdt WJ, Grünhagen DJ, van der Graaf WTA, Rueten-Budde AJ, Gelderblom H, et al. The role of perioperative chemotherapy in primary high-grade extremity soft tissue sarcoma: a risk-stratified analysis using PERSARC. European journal of cancer (Oxford, England : 1990). 2022;165:71-80.
34. Pasquali S, Pizzamiglio S, Touati N, Litiere S, Marreaud S, Kasper B, et al. The impact of chemotherapy on survival of patients with extremity and trunk wall soft tissue sarcoma: revisiting the results of the EORTC-STBSG 62931 randomised trial. European journal of cancer (Oxford, England : 1990). 2019;109:51-60.
35. Pasquali S, Palmerini E, Quagliuolo V, Martin-Broto J, Lopez-Pousa A, Grignani G, et al. Neoadjuvant chemotherapy in high-risk soft tissue sarcomas: A Sarculator-based risk stratification analysis of the ISG-STS 1001 randomized trial. Cancer. 2021.
36. van Praag VM, Rueten-Budde AJ, Jeys LM, Laitinen MK, Pollock R, Aston W, et al. A prediction model for treatment decisions in high-grade extremity soft-tissue sarcomas: Personalised sarcoma care (PERSARC). European journal of cancer (Oxford, England : 1990). 2017;83:313-23.
37. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Development and external validation of two nomograms to predict overall survival and occurrence of distant metastases in adults after surgical resection of localised soft-tissue sarcomas of the extremities: a retrospective analysis. The Lancet Oncology. 2016;17(5):671-80.
38. Issels RD, Lindner LH, Verweij J, Wessalowski R, Reichardt P, Wust P, et al. Effect of Neoadjuvant Chemotherapy Plus Regional Hyperthermia on Long-term Outcomes Among Patients With Localized High-Risk Soft Tissue Sarcoma: The EORTC 62961-ESHO 95 Randomized Clinical Trial. JAMA Oncol. 2018;4(4):483-92.
39. Kattan MW, Leung DH, Brennan MF. Postoperative nomogram for 12-year sarcoma-specific death. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(3):791-6.
40. Mariani L, Miceli R, Kattan MW, Brennan MF, Colecchia M, Fiore M, et al. Validation and adaptation of a nomogram for predicting the survival of patients with extremity soft tissue sarcoma using a three-grade system. Cancer. 2005;103(2):402-8.
41. Sampo M, Tarkkanen M, Tukiainen E, Popov P, Gustafson P, Lundin M, et al. A web-based prognostic tool for extremity and trunk wall soft tissue sarcomas and its external validation. Br J Cancer. 2012;106(6):1076-82.
42. Cahlon O, Brennan MF, Jia X, Qin LX, Singer S, Alektiar KM. A postoperative nomogram for local recurrence risk in extremity soft tissue sarcomas after limb-sparing surgery without adjuvant radiation. Ann Surg. 2012;255(2):343-7.
43. Smolle MA, Sande MV, Callegaro D, Wunder J, Hayes A, Leitner L, et al. Individualizing Follow-Up Strategies in High-Grade Soft Tissue Sarcoma with Flexible Parametric Competing Risk Regression Models. Cancers (Basel). 2019;12(1).
44. Sekimizu M, Ogura K, Yasunaga H, Matsui H, Tanaka S, Inagaki K, et al. Development of nomograms for prognostication of patients with primary soft tissue sarcomas of the trunk and extremity: report from the Bone and Soft Tissue Tumor Registry in Japan. BMC cancer. 2019;19(1):657.

45. Zhang SL, Wang ZM, Wang WR, Wang X, Zhou YH. Novel nomograms individually predict the survival of patients with soft tissue sarcomas after surgery. Cancer Manag Res. 2019;11:3215-25.
46. Xu Y, Xu G, Wu H, Lin F, Mao M, Baklaushev VP, et al. The Nomogram for Early Death in Patients with Bone and Soft Tissue Tumors. J Cancer. 2020;11(18):5359-70.
47. Tu Q, Hu C, Zhang H, Kong M, Peng C, Song M, et al. Development and Validation of Novel Nomograms for Predicting Specific Distant Metastatic Sites and Overall Survival of Patients With Soft Tissue Sarcoma. Technol Cancer Res Treat. 2021;20:1533033821997828.
48. Rueten-Budde AJ, van Praag VM, van de Sande MAJ, Fiocco M. Dynamic prediction of overall survival for patients with high-grade extremity soft tissue sarcoma. Surgical oncology. 2018;27(4):695-701.
49. Callegaro D, Miceli R, Bonvalot S, Ferguson PC, Strauss DC, van Praag VVM, et al. Development and external validation of a dynamic prognostic nomogram for primary extremity soft tissue sarcoma survivors. EClinicalMedicine. 2019;17:100215.
50. Rueten-Budde AJ, van Praag VM, van de Sande MAJ, Fiocco M. External validation and adaptation of a dynamic prediction model for patients with high-grade extremity soft tissue sarcoma. Journal of surgical oncology. 2021;123(4):1050-6.
51. Chibon F, Lagarde P, Salas S, Pérot G, Brouste V, Tirode F, et al. Validated prediction of clinical outcome in sarcomas and multiple types of cancer on the basis of a gene expression signature related to genome complexity. Nature medicine. 2010;16(7):781-7.
52. Chen S, Li N, Tang Y, Chen B, Fang H, Qi S, et al. Radiomics Analysis of Fat-Saturated T2-Weighted MRI Sequences for the Prediction of Prognosis in Soft Tissue Sarcoma of the Extremities and Trunk Treated With Neoadjuvant Radiotherapy. Front Oncol. 2021;11:710649.
53. Tian L, Zhang D, Bao S, Nie P, Hao D, Liu Y, et al. Radiomics-based machine-learning method for prediction of distant metastasis from soft-tissue sarcomas. Clin Radiol. 2021;76(2):158.e19-.e25.
54. Crombé A, Fadli D, Italiano A, Saut O, Buy X, Kind M. Systematic review of sarcomas radiomics studies: Bridging the gap between concepts and clinical applications? Eur J Radiol. 2020;132:109283.
55. Bertucci F, De Nonneville A, Finetti P, Perrot D, Nilbert M, Italiano A, et al. The Genomic Grade Index predicts postoperative clinical outcome in patients with soft-tissue sarcoma. Annals of oncology : official journal of the European Society for Medical Oncology. 2018;29(2):459-65.
56. Zhang B, Yang L, Wang X, Fu D. Identification of a survival-related signature for sarcoma patients through integrated transcriptomic and proteomic profiling analyses. Gene. 2021;764:145105.
57. Song Y, Yang K, Sun T, Tang R. Development and validation of prognostic markers in sarcomas base on a multi-omics analysis. BMC Med Genomics. 2021;14(1):31.
58. Eilber FC, Brennan MF, Eilber FR, Dry SM, Singer S, Kattan MW. Validation of the postoperative nomogram for 12-year sarcoma-specific mortality. Cancer. 2004;101(10):2270-5.
59. Squires MH, Ethun CG, Donahue EE, Benbow JH, Anderson CJ, Jagosky MH, et al. Extremity Soft Tissue Sarcoma: A Multi-Institutional Validation of Prognostic Nomograms. Annals of surgical oncology. 2022;29(5):3291-301.
60. Voss RK, Callegaro D, Chiang YJ, Fiore M, Miceli R, Keung EZ, et al. Sarculator is a Good Model to Predict Survival in Resected Extremity and Trunk Sarcomas in US Patients. Annals of surgical oncology. 2022.
61. Steyerberg EW. Clinical Prediction Models: A Practical Approach to Development, Validation, and Updating: Springer International Publishing; 2019.
62. Acem I, Martin E, van Houdt WJ, van de Sande MAJ, Grünhagen DJ, Verhoef C, et al. The Association of Metastasis Pattern and Management of Metastatic Disease with Oncological Out-

comes in Patients with Malignant Peripheral Nerve Sheath Tumors: A Multicenter Cohort Study. Cancers (Basel). 2021;13(20).
63. Casali PG, Abecassis N, Aro HT, Bauer S, Biagini R, Bielack S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2018;29(Suppl 4):iv51-iv67.
64. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making. 2006;26(6):565-74.
65. Vickers AJ, van Calster B, Steyerberg EW. A simple, step-by-step guide to interpreting decision curve analysis. Diagn Progn Res. 2019;3:18.
66. Krijkamp EM, Alarid-Escudero F, Enns EA, Jalal HJ, Hunink MGM, Pechlivanoglou P. Microsimulation Modeling for Health Decision Sciences Using R: A Tutorial. Med Decis Making. 2018;38(3):400-22.
67. Ferner RE, Gutmann DH. International consensus statement on malignant peripheral nerve sheath tumors in neurofibromatosis. Cancer Res. 2002;62(5):1573-7.
68. Vickers AJ, Kramer BS, Baker SG. Selecting patients for randomized trials: a systematic approach based on risk group. Trials. 2006;7:30.

PART IV

General Discussion, Future Perspectives and Summary

Chapter 12

General Discussion and Future Perspectives

General discussion

The premise of personalised medicine is effective care for each individual patient. (1) Therapeutic strategies and management should be tailored based on the unique characteristics of each individual. As a result, personalised medicine will endorse a sustainable and effective health system with less adverse events and higher quality of life. The Dutch Research agenda (Nationale Wetenschapsagenda, NWA), the Netherlands Federation of University Medical Centres and ZonNw underline the importance of personalised medicine through several research programs and taskforces. (1)

The core of personalised medicine centres on examining the diversity in clinical outcomes among patients, rather than focusing on the most basic common factor. Especially in the field of oncology large steps towards personalised medicine have been taken in the last decades. (2) This thesis aimed to contribute to a more personalised and patient-tailored approach in the management of soft tissue sarcoma (STS). We tried to achieve this goal by addressing the following three main questions:

1. **PART I:** Given the current practice,
   - what is the variation in clinical presentation and oncological outcome of patients with STS?
   - which factors influence this variation in oncological outcome?
2. **PART II:** How to better identify patients at risk and predict oncological outcome in patients with STS?
3. **PART III:** What is the current management of STS and how could prognostic tools play a role in the clinical decision making and management of STS?

An important application of personalised medicine is the use of prediction tools in the prevention, diagnosis, prognosis and treatment of cancer. In this thesis different steps of prediction research in patients with STS were undertaken. To critically appraise the relevance of prediction tools in the management of STS several aspects should be considered. First, we need to evaluate the variation in clinical outcomes and assess candidate predictors for prediction tools. Second, the validity of the tools should be considered. Last, the clinical relevance and possibilities for clinical implementation of the tools need to be evaluated. These three aspects are addressed subsequentially in the three parts of this thesis.

12.1 PART I: Risk factors and oncological outcome: heterogeneity within the sarcoma spectrum

The basis of prediction research is focussing on the variation in clinical outcome between patients instead of looking at the lowest common denominator. One of the first steps of developing prediction tools is selecting predictors. In **PART I** of this thesis several studies in patients with STS and malignant peripheral nerve sheath tumours (MPNSTs) were undertaken to assess clinicopathologic, treatment and patient-specific risk factors that explain variation or heterogeneity in oncological outcome. This thesis showed that some variation in oncological outcome could be explained by tumour specific characteristics such as grade, histological type, depth, tumour size and certain genetic alterations, e.g. alterations in the neurofibromatosis type 1 (NF1) and TP53 gene in MPNSTs (**chapter 3, 4** and **5**). Also, patient specific characteristics, such as age, were associated with differences in clinical presentation and oncological outcome.

Older age was independently associated with poorer survival, even after correcting for differences in tumour and treatment characteristics. In addition, elderly had a higher probability of developing distant metastasis (DM) in the first year after surgery compared with their younger counterparts with the same tumour and treatment characteristics (**chapter 2**). These findings suggest that elderly have a more aggressive tumour biology and/or a weaker tumour-specific immune response, which should be subject of future studies on aging and tumour biology.

Young adults presented more often with 'whoops'-surgery (**chapter 2**). 'Whoops'-surgery is a surgical procedure without appropriate diagnosis, preoperative imaging and planning as the tumour mass was assumed to be benign, but the final pathological diagnosis after surgery showed a malignant tumour. This suggests that young adults are more vulnerable to incorrect diagnosis and treatment delay (3), which might result in poorer functional and oncological outcome. To prevent treatment delay, medical professionals must be aware that sarcoma can affect patients of all age groups. In addition, a clear patient referral protocol or non-invasive diagnostic tool with high sensitivity might help medical professionals to differentiate between suspected and unsuspected soft tissue tumours and refer timely. (4-6)

12.1.1 Future perspectives for studies on risk factors

As in this thesis, most of the observational studies in STS focus on oncological outcome and associated clinical risk factors. However, studies on genetic and epigenetic variations can play an important role in personalised medicine, as demonstrated in gastrointestinal stromal tumours (GIST); a selected group of patients with GIST

receive targeted therapy based on genetic mutations in the KIT proto-oncogene on exon 9 and 11. (7) The determination of genetic and epigenetic variations in STS could identify novel molecular targets for targeted therapies and improve diagnostic and prognostic ability in STS. Although molecular diagnostics in STS has expanded rapidly in recent years, prognostic tools are still mainly based on clinical risk factors. Future studies should be encouraged to assess the added value of genetic and epigenetic variations in existing prediction tools. This could be done by extending (inter)national cohorts, such as MONACO, with pathological and molecular data by linking the patients in the cohort with the Public Pathology Database, PALGA. The added value of the identified immunohistochemical markers and genetic variations of **chapter 3** could be further evaluated in this cohort. These studies should focus on the right balance between prognostic ability and clinical usability, as not all prognostic biomarkers would be readily available in clinical practice.

12.2 PART II: DEVELOPMENT OF PREDICTION TOOLS IN STS: IDENTIFYING PATIENTS AT RISK AND PREDICTING ONCOLOGICAL OUTCOME

Prediction tools are instruments which are commonly used to estimate the prognosis or diagnosis for individual patients. Prediction tools generate individual probabilities of a certain clinical event or diagnosis incorporating multiple risk factors. In prediction research two types of tools exist:

1. **Diagnostic tools** try to answer the question 'does this patient have the disease?' A diagnostic tool tries to answer a cross-sectional question. A patient presents with symptoms. Based on predictors, e.g. patient characteristics, imaging tests, laboratory test or other tests, we try to determine whether the patient has the disease or not. The central question for the performance of a diagnostic tool is how well the tool could discriminate between individuals with and without the disease. Measures that are commonly used to assess the diagnostic accuracy or discriminative ability of a diagnostic tool are c-index, sensitivity, specificity, positive and negative predictive values and likelihood ratios. (8)

2. **Prognostic tools** try to answer questions in the future. These are longitudinal questions. Prognostic tools try to estimate the probability that a patient develops a certain event or condition at a specified time point in the future. Often, these tools are used to inform the physician and patient in the medical decision making for certain (additional) treatments. Good calibration of the tool is essential in this case. Measures that are commonly used to assess the prognostic ability or

performance of a prognostic tool are C-index, observed/expected-ratio and calibration plots. Prognostic tools that try to predict treatment response are called predictive tools in literature.

In **PART II** of this thesis both types of prediction tools were developed for different clinical applications. In the next paragraphs the validity and clinical relevance of both tools will be appraised.

12.2.1 A minimal-invasive diagnostic tool: the eNose

In **chapter 6** the discriminative ability of a new molecular fingerprint based on exhaled breath, the eNose, was assessed in patients with and without STS. This proof-of-concept study yielded an excellent discriminative ability with a high sensitivity and good specificity, suggesting that the eNose could become a promising minimal invasive diagnostic tool for STS. However, as this pilot study had a relatively small sample size and only internal cross-validation was performed, the validity of the eNose should be further assessed in larger external cohorts of patients. In addition, the clinical usability and relevance should be further evaluated in an implementation study, before this diagnostic tool could be used as standard practice.

The eNose has in potential large clinical relevance, as differentiating between benign soft tissue tumours and STS is challenging in clinical practice. It has been estimated that benign soft tissue tumours occur 300 times more often than their malignant counterparts. (9-11) As a result, "whoops"-surgeries with inadequate surgical margins are relatively frequently performed in STS. (10, 12-17) The eNose could achieve a higher pre-test probability for STS and potentially reduce the number of "whoops"-surgeries, re-excisions and biopsies in patients with soft tissue tumours. For this clinical application, biopsies should still be performed as gold standard for diagnosing STS. The eNose could be used to decide which patients should get a referral to a tertiary sarcoma centre for biopsy. In this case, maximizing the sensitivity of the tool, to minimize the number of false negatives, is clinically most desirable. Especially, in young patients with small and superficial STS the rate of "whoops"-excisions is relatively high. (12, 14-17) Therefore, the use of the eNose seems most promising in this target population.

12.2.2 A type-specific prognostic tool: MONACO

In **chapter 7** of this thesis we developed and validated a type-specific prognostic tool, the MONACO prediction tool, for overall survival (OS). Patients with MPNST who underwent macroscopically complete surgical resection with curative intent from

eleven international sarcoma centres were included in this observational cohort study. In addition, we compared the validity of the MONACO tool with two widely used prognostic tools for soft tissue sarcoma of the extremities (eSTS), Sarculator and PERSARC. (18, 19) The MONACO tool included MPNST-specific predictors and was applicable for a wider range of patients compared with Sarculator and PERSARC. As a result, the MONACO tool had a better performance than Sarculator and PERSARC in patients with MPNST. These results suggest that the MONACO tool better predicts OS when compared with the generic tools Sarculator and PERSARC.

However, although internal-external cross-validation qualifies as external validation, the MONACO tool may benefit from further validation. (20) As reflected in the internal-external cross-validation of this study, model performance differs to some extent across centres or regions, due to case-mix. Ideally, prediction tools should be updated for each centre or region to improve local validity, if large regional differences exist. (21) In addition, prediction tools can become less valid with time due to various reasons, such as improvement in treatment strategies and screening tools. (22) With this study, we aimed to stimulate researchers and physicians to update the MONACO model with time or setting-specific estimates through Evidencio, a medical prediction platform that allows for easy and quick model validation. In addition, all estimates have been published online to validate, update, or incorporate the estimated predictors in existing or future prediction tools.

Although the model performance of the MONACO tool seems reasonably good, good model performance does not necessarily imply clinical relevance. (23) To assess whether a model can be beneficial in clinical practice to guide decision making on therapy or surveillance, we need a threshold probability for such decisions. (24) The decision curve analysis showed that the MONACO tool had a higher net benefit when used for treatment indication compared with treating all or none of the patients, across all reasonable threshold probabilities. Therefore, the MONACO tool could be of added value as a clinical prediction tool.

12.2.3 Future perspectives for prediction research

In an era of personalized medicine, the popularity of prediction tools has been steadily increasing. This has also been reflected by the exponential growth of medical papers that develop or validate prediction tools. Novel diagnostic tools such as machine-learning models based on magnetic resonance imaging (MRI) or computed tomography (CT) (radiomics), molecular fingerprints (e.g. eNose or pathomics) and liquid biopsies (from blood or urine), hold large potential as new diagnostic biomarkers in clinical practice within oncology. (25-29) In addition, these diagnostic

biomarkers might also hold a prognostic and predictive value. (30, 31) However, the implementation into clinical practice is lagging behind. An important reason is the lack of external validation of these kind of studies. There is a high need for large multicentre datasets including clinical, molecular, imaging and patient-reported data, which could validate and update the existing omics tools. International cohort studies, such as the MONACO consortium, might be a good starting point to further build a high performing infrastructure for the collection of these kind of multi-omics data.

Prognostic tools in STS, such as Sarculator and PERSARC, are gradually finding their place in clinical practice. To ensure validity and clinical relevance, prediction tools should be constantly updated for differences in time, clinical setting and patient population. (24) With the MONACO prediction tool, we aimed to ensure the validity in the MPNST population and hope that these estimates will be incorporated in the existing tools. To further improve prognostication of patients with MPNST, other clinically relevant outcomes such as the risk on local recurrence (LR) and DM could be implemented in the tool. In addition, dynamic models allowing for time-varying variables which allow prediction of prognosis at various time points during follow-up, might further improve validity and clinical relevance. Future studies are needed to further evaluate the clinical relevance and clinical applicability of the MONACO tool for clinical-decision making.

12.3 PART III: THE MANAGEMENT OF SOFT TISSUE SARCOMA: FROM ONE-SIZE FITS ALL TO PATIENT-TAILORED MEDICINE

Clinical relevance goes beyond predicting prognosis. Prediction tools that have specific diagnostic or therapeutic consequences, such as the Ottawa Ankle Rules to determine the need for X-rays in patients with ankle injuries (32) and the Padua Prediction Score to assess which hospitalized patients should receive thromboprophylaxis (33) achieved large clinical impact and are widely used across the world.

Chapter 8 provided an insight into clinical decision-making of sarcoma specialist in an international cross-sectional study. This study showed that 81% of the sarcoma specialists would consider the use of a prediction tool for the indication of perioperative chemotherapy (CTX) in patients with primary resectable eSTS. Perioperative CTX is not the standard of care and is only reserved for a selected group of high-risk patients with primary STS. At that time the clinical guideline recommended that perioperative CTX can be proposed as an option to high-risk individuals. A high-risk individual was defined as a patient with a high-grade (FNCLCC grade II or III) deep-seated tumour of more than 5 cm. (34) During my PhD programme, the

clinical guidelines changed. Still, perioperative CTX is not standard of care but now the clinical guidelines recommend the use of prediction tools to identify patients with high risk of death. (35)

The use of perioperative CTX in the management of STS is a widely debated topic among sarcoma experts. However, there is a consensus on the standard chemotherapeutic regimen, namely anthracyclin in combination with ifosfamide. To date, five randomised controlled trials (RCTs) have compared the effect of perioperative AI-based CTX with no CTX in patients with localized STS. (36-40) Three RCTs stopped prematurely because of poor patient accrual. (37, 39, 40) The largest and most recent trial (EORTC 62931) yielded no significant effect of perioperative CTX on OS in patients with localized STS. (36) However, in this trial 24% of the patients had a tumour smaller than 5 cm and 40% had a low or intermediate grade tumour, which are considered low-risk patients. (34) In **chapter 9** we aimed to assess the effect of perioperative AI-based CTX on OS in the largest multicentre cohort to date of more than 5500 patients with primary high-grade resectable eSTS. We stratified the patients into three risk groups based on the PERSARC prediction tool. We used inverse probability of treatment weighting (IPTW) to balance baseline characteristics in the exposed and unexposed group. This study found a clinically and statistically significant effect of CTX in a selected group of high-risk patients with a predicted 5-year OS of ≤66%, with an absolute survival benefit of 11%. Beside the prognostic value of PERSARC, this study suggests that prediction tools could also be used as predictive tool for the indication of perioperative CTX. **Chapter 11** demonstrated that the PERSARC tool is also clinically relevant across a wide range of threshold probabilities, including a threshold probability of OS of ≤66%. These studies highlight the potential impact of prediction tools in the management of STS in a patient-tailored manner.

12.3.1 Future perspectives of patient-tailored management of STS

Although the increasing evidence that perioperative CTX can play a role in a selected group of high-risk patients with primary STS, perioperative CTX is only offered in a few centres across the world for this patient population. New RCTs assessing the effect of perioperative CTX in STS seem not feasible as RCTs are costly and time-consuming. RCTs in STS require multicentre collaborations as the number of patients in large scare sarcoma centres are still too low to complete a RCT in a reasonable time frame. In addition, an increasing number of sarcoma experts do not believe in equipoise, which makes randomizing patients unethical. Therefore, only large scale well-designed prospective multicentre cohort studies, considering all possible actions that could introduce bias and influence causal inference, seem feasible. In **paragraph 12.5**, I will further elaborate on causal inference in observational studies.

Besides the clinical application of prediction tools to identify patients that benefit of perioperative CTX, prediction tools could also be used to tailor patient follow-up after primary treatment. **Chapter 8** demonstrated a wide variation in the frequency of follow-up visits and follow-up modalities. Also, clinical guidelines do not provide a clear follow-up schedule for patients with curatively treated primary STS. (35, 41) Future studies should explore the use of prognostic tools for patient-tailored follow-up regimen. Both, Sarculator and PERSARC provide dynamic predictions. (42, 43) PERSARC allows to predict the risk of LR and DM at different time points during follow-up. These predictions could be used to assess whether additional imaging is needed and what imaging modality should be used. It seems reasonable that if a patient has a low risk of disease progression, no imaging is needed; if a patient has a high risk of LR, the patient should get an MRI scan of the primary tumour site; and if a patient has a high risk of DM, the patient should get a chest CT scan or chest X-ray. However, at which risk threshold imaging should be considered, remains subject of debate and research.

12.4 Future perspectives

In the discussion above, multiple possibilities and research suggestions were presented to enhance the management of STS in a more patient-tailored manner. In this paragraph, I summarise them and add more general reflections and recommendations.

12.4.1 Towards patient-tailored management

When to treat?

Prediction models have shown to be valuable tools to identify the patients that benefit most from a certain treatment. The assessment of this heterogeneity in treatment effect should be ideally performed in an RCT in which the treatment of interest is randomised. (44) A first step to assess whether a prediction tool could be used in the decision-making process for a certain treatment, is to perform a decision curve analysis. (23) However, to assess at which threshold a patient should receive the treatment, a more extensive risk-benefit or cost-effectiveness analysis should be performed weighting all benefits, harms and costs related to the treatment. For the indication of perioperative CTX in patients with primary resectable STS, no such analyses have been conducted and should be subject of future research. In addition, the use of prediction tools for the indication perioperative RTX should be elaborated in future studies. The Memorial Sloan Kettering Cancer Centre developed a prediction tool for the risk of local recurrence after limb-sparing surgery without RTX. (45) However, the counterfactual, namely, the (reduced) risk of local recurrence after limb-sparing surgery with RTX and, thus, the net treatment benefit of RTX, remains unclear.

Ensuring validity and usability

As set forth in **paragraph 12.2.1** prediction tools require regular updates to ensure validity. Ideally, prediction tools should be frequently updated, extended and validated with new predictors and for new settings (**Figure 1**). This dynamic circle comes with its own set of challenges.

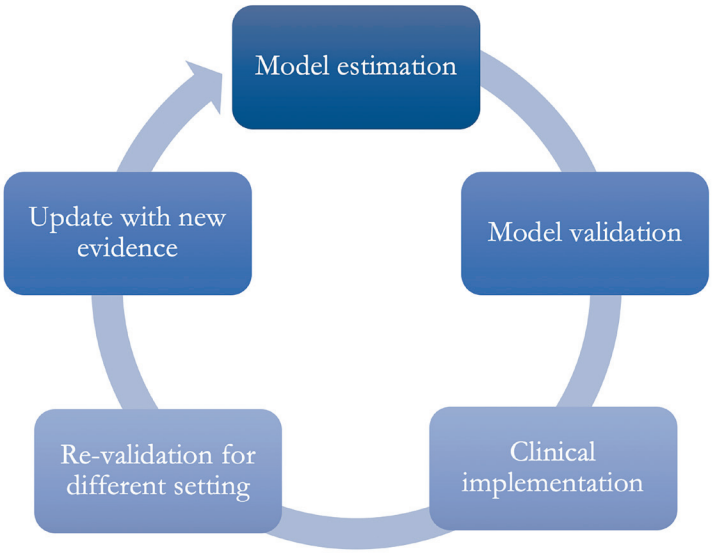

Figure 1. Dynamic circle of the development of prediction tools

First, prediction tools should provide all key details about the development and specification of the prediction tool. Only then other researchers can replicate, validate, update or extend the model for their own settings, taking into account different case-mixes. Unfortunately, many published prediction tools in STS lack crucial information such as the model intercept or baseline hazard at the predicted time points, hampering the circle for valid models.

Second, dynamic updating requires large-scale high-quality data. Prediction models are only as good as their data. (46) In prediction research the main concerns are selection and misclassification bias, restricting internal validity, as well as overfitting, restricting external validity. In addition, prediction tools have the potential to worsen pre-existing inequalities which are inherent to the current healthcare system, such as racial bias. (47) A well-designed study with a good infrastructure for data collection and integration is required as prediction models are becoming more complex and increasingly important in the delivery of healthcare.

Last, model extension should focus on the right balance between validity and usability. Especially, with the increasing interest in molecular research in STS, (epi)genetic risk factors are becoming increasingly abundant in literature. However, in clinical practice these risk factors are not (yet) widely examined owing, for example, to the costs of these tests. This also applies to new technologies and multi-omics data. Hence, risk factors that are not easily available in clinical practice, should only be considered in prediction models if the risk factor substantially improves the prognostic ability and has therapeutic consequences.

The added value of artificial intelligence

The eNose, introduced in **chapter 6** of this thesis, is one of the many examples of new minimal-invasive diagnostic tools using a molecular fingerprint and artificial intelligence to detect diseases. Other advances in diagnostic research are the use of liquid biopsies or information collected from wearables to predict the probability of a certain disease. These advances have not yet made their mark in clinical practice in the field of STS and other types of cancer. An important reason for the lack of clinical impact of these diagnostic advances is the rarity of STS, complicating large prospective studies to further improve and validate these tools.

The improvement and validation of the diagnostic ability of existing diagnostic modalities in combination with artificial intelligence, require less prospective data as these diagnostic tools have been used for years. The added value of the use of artificial intelligence on these diagnostic tools, e.g. MRI, CT (radiomics) and pathological slides (pathomics), could, at least partially, be assessed retrospectively. The use of artificial intelligence on existing diagnostic modalities could therefore be one of the first applications of artificial intelligence in the diagnostic work-up of patients with STS. However, the same concerns hold as stated in the preceding section.

12.4.2 Towards patient-tailored follow-up

The application of prediction tools to guide multimodality therapy is gradually gaining acceptance in the management of STS. Another potentially valuable application of prediction tools may lie in the optimization of follow-up strategies. There is a lack of consensus on the optimal follow-up schedule and diagnostic method for disease progression in patients with STS. (48, 49) Dynamic prediction tools could provide guidance to sarcoma specialist regarding the necessary follow-up frequency and the most suitable diagnostic modality depending on the risk of disease progression at different time points during follow-up. This may lead to a patient-tailored follow-up regimen, preventing unnecessary imaging and anxiety in patients with a low risk of disease progression while ensuring timely diagnosis for those at higher risk.

14.4.3 Towards patient-centred decisions

Besides the application of prediction tools for patient-tailored treatment strategies and follow-up schedules, prediction tools might also inform patients about their prognosis and increase patients' understanding about risks and prognosis. This could improve the shared decision-making process and satisfaction of patients. Nonetheless, it remains unclear whether prediction tool-assisted decisions improve patients' understanding and patient outcomes. (22) VALUE PERSARC is a multicentre randomised study that aims to evaluate whether the use of a prediction tool improves patients' informed decision making and satisfaction. (50) This study will shed new light on whether decision supporting interventions such as the use of a prediction tool improve patient outcomes.

Another upcoming field of research is the use of personality information in prediction tools to improve personality-tailored decisions. This field of research could identify problems from patient's perspective and develop strategies that may improve patient's experience and satisfaction. Essential is the standardization of personality assessment tools ensuring reliability and consistent risk scores.

12.5 Making the most of large observational data

Because of the rarity and heterogeneity of STS, there are significant challenges associated with conducting RCTs. Therefore, most studies in the field of STS are based on real-world observational data. This thesis illustrates thoroughly how observational studies play an important role in the identification of risk factors, development of prediction tools and causal inference. However, observational studies are prone to systemic errors or biases that hamper our ability to make (causal) conclusions based on these studies.

12.5.1 Bias

These systemic errors could be clustered into three types of biases:

1. **Selection bias** refers to the difference in participants and non-participants between the exposed and unexposed group which is related to the outcome. This could occur when the selection of participants is not a random process, for example in case of self-selection. Also, selective non-response and selective loss-to-follow up, which is related to both exposure and outcome, could introduce selection bias. (51) Selection bias could be introduced for example, when patients are referred to a tertiary centre only if the patient is at high risk for a certain outcome or

if the patient wishes to receive a certain treatment (irrespective of the risk on outcome). If we intend to conduct a study in this tertiary centre aiming to assess the effect of this treatment, we should be aware that patients who did not receive the treatment are at high risk of the outcome, while patients who did receive the treatment could have a lower risk of the outcome because of the referral policy. This referral policy introduces therefore selection bias and makes the exposed and unexposed group not comparable or exchangeable.

2. **Confounding bias** occurs when a third variable (confounder) is associated with both exposure and outcome, leading to a distortion in the observed relationship between the exposure and outcome. (52) For example, RTX is usually offered to patients with a large tumour. These patients usually have a poorer prognosis than patients with a small tumour. If we do not account for tumour size in our analysis, we would mistakenly conclude that RTX leads to poorer prognosis, while tumour size is the confounding factor distorting the relationship between RTX and survival.

3. **Information bias** arises when exposure or outcome are measured differently in the compared groups. This would have been the case when patients who got CTX receive more frequent chest imaging for the detection of DM during follow-up than patients who did not receive CTX. The higher frequency of imaging could increase the detection rate of DM. Other sources of information bias are recall bias or non-random misclassification.

In a randomized controlled trial, these systematic errors are typically minimized or eliminated by design, enabling us to draw causal inferences from such studies. At the entry of a trial, there is no selection bias as exposure status is determined by a randomization process, thus preventing differences between participants and non-participants related to exposure. Successful randomization also eliminates confounding bias, and information bias could be ruled out by blinding the patient, physician and investigators. However, still in RCTs bias could be introduced due to e.g. inadequate allocation concealment, selective loss to follow-up, or inability to blind. (53, 54)

12.5.2 Causal inference in observational studies

In observational studies the aforementioned systematic errors are rarely ruled out by design, especially in retrospective studies. Therefore, it is crucial to take these biases into account in the study design and analysis plan.

Selection bias could be minimized by ensuring that the exposed and unexposed participants included in the study do not differ based on factors that are related to the outcome. In the aforementioned example of selection bias, patients not referred to the tertiary centre should also be included to minimize the bias.

Information bias could be minimized by standardized surveillance protocols and diagnostic tools that are irrespective of exposure. Furthermore, hard outcomes such as mortality or amputation rates are less susceptible to information bias than for example DM rate. The latter is dependent on the frequency of surveillance, which could be associated with the exposure. Hence, employing such hard outcomes could minimize bias.

Confounding bias is arguably the largest source of bias in causal inference studies in observational data. There is a wide variety of statistical methods available to address confounding bias in observational studies such as stratification, (Directed Acyclic Graph-guided) multivariable regression analysis, propensity score based methods such as matching or weighting, and instrumental variable approach. (55-57) To consider all confounding factors adequately, a profound understanding of the subject matter is essential when applying these statistical methods. Incomplete data collection makes it impossible to achieve full adjustment for confounding bias (except for instrumental variable analysis), commonly referred to as residual confounding. In **chapter 9** we used a combination of propensity score weighting, previously referred as IPTW, and stratification. IPTW was used to balance the differences in baseline covariates between patients who received CTX and those who did not. This was achieved by reweighting the study population according to their propensity scores, thereby creating a pseudo-population where the treatment assignment was independent of the observed covariates. (58) Stratification based on the PERSARC scores was used to assess the impact of perioperative CTX in various risk groups, evaluating the heterogeneity in treatment effect. **Part I** and **II** of this thesis primarily involved predictive research, in which confounding bias does not play a role and no adjustments are needed to control for this bias.

12.5.3 Missing data

An often-overlooked source of bias is missing data in observational studies. In this thesis we mainly handled missing values using multiple imputation techniques. Most statistical software programs usually handle missing data including only complete cases in the analysis, often leading to selection bias. (59) Complete case analysis is only valid if missing data is completely at random. (59, 60) This implies that there is no systematic difference between the missing and observed data, which is rarely the

case in observational data. (59) Complete case analysis in datasets with other types of missing data will result in invalid and imprecise outcomes. Hence, it is crucial to handle missing data appropriately to improve the validity of the results and maximize the statistical power of the data analyses. (60)

Such bias caused by missing data can be overcome using multiple imputation methods that allow individuals with incomplete data to be included in the analysis. These methods take uncertainty about the missing data into account by creating multiple datasets with different plausible imputed values, under the assumption of missing at random. (59, 61) This assumption implies that any systematic difference between the missing and observed data can be explained by observed values in the dataset. (61) This imputation method usually leads to more valid and precise outcomes compared with complete-case analysis or single imputation methods. (59, 61)

12.5.4 Collaborative efforts

In this thesis we tried to mitigate aforementioned sources of bias in observational studies in several ways. These include the utilization of only well-defined variables and hard outcomes, the application of several statistical methods to account for confounders, and the use of multiple imputation techniques to handle missing values.

However, observational studies offer also multiple advantages in comparison with randomized trials, that underscore their importance in the research field of STS. This thesis, for example, could not have existed without the multiple multicentre collaborations. In addition, multicentre cohort studies do not only increase sample size, improve generalizability, and allow to answer a wider range of research questions but also offer multiple benefits beyond sheer data expansion. These collaborations can act as a catalyst for standardized data collection protocols and quality control measures. Additionally, these collaborations enhance efficiency by sharing the burden of data collection and administrative tasks, and provide an infrastructure for cohort embedded trials, integrating clinical interventions within an existing well-characterized cohort framework. Lastly, these collaborations facilitate long-term research, benefiting from their established infrastructure and enduring relationship with the participating centres.

In the Netherlands, the management of STS has been centralized in eight referral sarcoma centres. These centres have a collective work group, the Dutch Sarcoma Group, in which all Dutch sarcoma specialists are united to improve the quality of care of patients with STS. This concentration of patients and expertise can lead to improved patient care and more effective research. In the last years, an initiative has

started to build a multicentre infrastructure in The Netherlands for data collection of patients with STS. This collaboration aims to standardize management and collection protocols to ensure a high-quality sarcoma registry.

12.5.5 Future perspectives for the Dutch sarcoma registry

The Dutch sarcoma registry collaboration holds significant potential and could facilitate future research objectives. I believe that a few key factors capable of enhancing this potential include the utilization of technology and artificial intelligence in the data collection process, as well as the integration of patient accrual and follow-up into daily clinical practice. First of all, it is crucial to standardize data management using predefined variables and definitions, and to collect patient data efficiently and securely within an electronic data capture system. Electronic health record systems should be linked with the data capture system with the help of artificial intelligence and text analytics. A human-in-the-loop will ensure data reliability. This data linkage will enable the gathering of clinical data, such as baseline, tumour- and treatment-related characteristics.

In addition, patient selection and follow-up should be integrated into daily clinical practice upon entry to ensure that all patients are informed about the registry and that patient-reported outcomes are included in the registry. Within clinical practice, sarcoma specialists should reflect on the patient-reported outcomes to improve the quality of personalized care, resulting in increased response rates as a favourable side effect. Ideally, computer adaptive questionnaires should be integrated into the registry to enable personalized and efficient questionnaires.

A well-established infrastructure of centralized care and data collection allows for a reliable view on nationwide care and outcomes of STS, but, perhaps more importantly, allows for new prospective (intervention) studies embedded within the sarcoma registry. These studies can, for instance, evaluate the effectiveness of a new therapy in a specific subgroup of patients or to assess the prognostic value of a promising new biomarker in blood or other tissues. Once established, the registry infrastructure could be relatively easily extended to other sarcoma centres worldwide.

12.6 Concluding remarks

In an era of personalized medicine, this thesis aimed to contribute to a more personalised approach in the management of STS. I hope that this work has illustrated how prediction tools enable the shift from a one-size-fits-all approach to patient-tailored management of STS. Prediction tools have proven their worth by identifying high-

risk patients who might benefit from (neo)adjuvant CTX. Furthermore, these tools might facilitate patient-tailored follow-up strategies and patient-centred decisions.

Continued refinement of existing tools, incorporating promising new predictors, and re-calibrating and re-validating them for different clinical settings is essential to ensure a good application of these tools in clinical practice. Future research should focus on the clinical implementation of these tools and their clinical consequences. Multicentre collaborative efforts such as the Dutch Sarcoma Group are of great importance to further enhance large-scale high-quality data of patients with STS to further optimize the patient-tailored care of STS.

References

1. Sleijfer SC, I. Hasselaar, J. Meijer, I. Kennisagenda Personalised Medicine. Houten Drukkerij Badoux 2018.
2. Ciardiello F, Arnold D, Casali PG, Cervantes A, Douillard JY, Eggermont A, et al. Delivering precision medicine in oncology today and in future-the promise and challenges of personalised cancer medicine: a position paper by the European Society for Medical Oncology (ESMO). Annals of oncology : official journal of the European Society for Medical Oncology. 2014;25(9):1673-8.
3. Younger E, Husson O, Bennister L, Whelan J, Wilson R, Roast A, et al. Age-related sarcoma patient experience: results from a national survey in England. BMC cancer. 2018;18(1):991.
4. Buvarp Dyrop H, Vedsted P, Rædkjær M, Safwat A, Keller J. Routes to Diagnosis for Suspected Sarcoma: The Impact of Symptoms and Clinical Findings on the Diagnostic Process. Sarcoma. 2016;2016:8639272.
5. Nakamura T, Matsumine A, Matsubara T, Asanuma K, Uchida A, Sudo A. The symptom-to-diagnosis delay in soft tissue sarcoma influence the overall survival and the development of distant metastasis. Journal of surgical oncology. 2011;104(7):771-5.
6. Ferrari A, Miceli R, Casanova M, Meazza C, Favini F, Luksch R, et al. The symptom interval in children and adolescents with soft tissue sarcomas. Cancer. 2010;116(1):177-83.
7. Casali PG, Blay JY, Abecassis N, Bajpai J, Bauer S, Biagini R, et al. Gastrointestinal stromal tumours: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2022;33(1):20-33.
8. Šimundić AM. Measures of Diagnostic Accuracy: Basic Definitions. Ejifcc. 2009;19(4):203-11.
9. Kang S, Han I, Lee SA, Cho HS, Kim HS. Unplanned excision of soft tissue sarcoma: the impact of the referring hospital. Surgical oncology. 2013;22(2):e17-22.
10. Fiore M, Casali PG, Miceli R, Mariani L, Bertulli R, Lozza L, et al. Prognostic effect of re-excision in adult soft tissue sarcoma of the extremity. Annals of surgical oncology. 2006;13(1):110-7.
11. Tedesco NS, Henshaw RM. Unplanned Resection of Sarcoma. J Am Acad Orthop Surg. 2016;24(3):150-9.
12. Smolle MA, Tunn PU, Goldenitsch E, Posch F, Szkandera J, Bergovec M, et al. The Prognostic Impact of Unplanned Excisions in a Cohort of 728 Soft Tissue Sarcoma Patients: A Multicentre Study. Annals of surgical oncology. 2017;24(6):1596-605.
13. Chandrasekar CR, Wafa H, Grimer RJ, Carter SR, Tillman RM, Abudu A. The effect of an unplanned excision of a soft-tissue sarcoma on prognosis. J Bone Joint Surg Br. 2008;90(2):203-8.
14. Nakamura T, Kawai A, Hagi T, Asanuma K, Sudo A. A comparison of clinical outcomes between additional excision after unplanned and planned excisions in patients with soft-tissue sarcoma of the limb : a propensity matching cohort study. The bone & joint journal. 2021;103-b(12):1809-14.
15. Zaidi MY, Ethun CG, Liu Y, Poultsides G, Howard JH, Mogal H, et al. The impact of unplanned excisions of truncal/extremity soft tissue sarcomas: A multi-institutional propensity score analysis from the US Sarcoma Collaborative. Journal of surgical oncology. 2019;120(3):332-9.
16. Charoenlap C, Imanishi J, Tanaka T, Slavin J, Ngan SY, Chander S, et al. Outcomes of unplanned sarcoma excision: impact of residual disease. Cancer Med. 2016;5(6):980-8.
17. Liang Y, Guo TH, Xu BS, Hong DC, Qiu HB, Zhou ZW, et al. The Impact of Unplanned Excision on the Outcomes of Patients With Soft Tissue Sarcoma of the Trunk and Extremity: A Propensity Score Matching Analysis. Front Oncol. 2020;10:617590.

18. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Development and external validation of two nomograms to predict overall survival and occurrence of distant metastases in adults after surgical resection of localised soft-tissue sarcomas of the extremities: a retrospective analysis. The Lancet Oncology. 2016;17(5):671-80.
19. van Praag VM, Rueten-Budde AJ, Jeys LM, Laitinen MK, Pollock R, Aston W, et al. A prediction model for treatment decisions in high-grade extremity soft-tissue sarcomas: Personalised sarcoma care (PERSARC). European journal of cancer (Oxford, England : 1990). 2017;83:313-23.
20. Steyerberg EW, Harrell FE, Jr. Prediction models need appropriate internal, internal-external, and external validation. J Clin Epidemiol. 2016;69:245-7.
21. Binuya MAE, Engelhardt EG, Schats W, Schmidt MK, Steyerberg EW. Methodological guidance for the evaluation and updating of clinical prediction models: a systematic review. BMC Med Res Methodol. 2022;22(1):316.
22. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP. Nomograms in oncology: more than meets the eye. The Lancet Oncology. 2015;16(4):e173-80.
23. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making. 2006;26(6):565-74.
24. Steyerberg EW. Clinical Prediction Models: A Practical Approach to Development, Validation, and Updating: Springer International Publishing; 2019.
25. Aerts HJ. The Potential of Radiomic-Based Phenotyping in Precision Medicine: A Review. JAMA Oncol. 2016;2(12):1636-42.
26. Coudray N, Ocampo PS, Sakellaropoulos T, Narula N, Snuderl M, Fenyö D, et al. Classification and mutation prediction from non-small cell lung cancer histopathology images using deep learning. Nature medicine. 2018;24(10):1559-67.
27. Cescon DW, Bratman SV, Chan SM, Siu LL. Circulating tumor DNA and liquid biopsy in oncology. Nat Cancer. 2020;1(3):276-90.
28. Vos M, Starmans MPA, Timbergen MJM, van der Voort SR, Padmos GA, Kessels W, et al. Radiomics approach to distinguish between well differentiated liposarcomas and lipomas on MRI. Br J Surg. 2019;106(13):1800-9.
29. Zhang M, Tong E, Hamrick F, Lee EH, Tam LT, Pendleton C, et al. Machine-Learning Approach to Differentiation of Benign and Malignant Peripheral Nerve Sheath Tumors: A Multicenter Study. Neurosurgery. 2021;89(3):509-17.
30. Skrede OJ, De Raedt S, Kleppe A, Hveem TS, Liestøl K, Maddison J, et al. Deep learning for prediction of colorectal cancer outcome: a discovery and validation study. Lancet. 2020;395(10221):350-60.
31. Feng L, Liu Z, Li C, Li Z, Lou X, Shao L, et al. Development and validation of a radiopathomics model to predict pathological complete response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer: a multicentre observational study. Lancet Digit Health. 2022;4(1):e8-e17.
32. Stiell IG, Greenberg GH, McKnight RD, Nair RC, McDowell I, Worthington JR. A study to develop clinical decision rules for the use of radiography in acute ankle injuries. Annals of emergency medicine. 1992;21(4):384-90.
33. Barbar S, Noventa F, Rossetto V, Ferrari A, Brandolin B, Perlati M, et al. A risk assessment model for the identification of hospitalized medical patients at risk for venous thromboembolism: the Padua Prediction Score. Journal of thrombosis and haemostasis. 2010;8(11):2450-7.
34. Casali PG, Abecassis N, Aro HT, Bauer S, Biagini R, Bielack S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2018;29(Suppl 4):iv51-iv67.

35. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2021;32(11):1348-65.
36. Woll PJ, Reichardt P, Le Cesne A, Bonvalot S, Azzarelli A, Hoekstra HJ, et al. Adjuvant chemotherapy with doxorubicin, ifosfamide, and lenograstim for resected soft-tissue sarcoma (EORTC 62931): a multicentre randomised controlled trial. The Lancet Oncology. 2012;13(10):1045-54.
37. Fakhrai N, Ebm C, Kostler WJ, Jantsch M, Abdolvahab F, Dominkus M, et al. Intensified adjuvant IFADIC chemotherapy in combination with radiotherapy versus radiotherapy alone for soft tissue sarcoma: long-term follow-up of a prospective randomized feasibility trial. Wiener klinische Wochenschrift. 2010;122(21-22):614-9.
38. Frustaci S, Gherlinzoni F, De Paoli A, Bonetti M, Azzarelli A, Comandone A, et al. Adjuvant chemotherapy for adult soft tissue sarcomas of the extremities and girdles: results of the Italian randomized cooperative trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(5):1238-47.
39. Gortzak E, Azzarelli A, Buesa J, Bramwell VH, van Coevorden F, van Geel AN, et al. A randomised phase II study on neo-adjuvant chemotherapy for 'high-risk' adult soft-tissue sarcoma. European journal of cancer (Oxford, England : 1990). 2001;37(9):1096-103.
40. Petrioli R, Coratti A, Correale P, D'Aniello C, Grimaldi L, Tanzini G, et al. Adjuvant epirubicin with or without Ifosfamide for adult soft-tissue sarcoma. Am J Clin Oncol. 2002;25(5):468-73.
41. Von Mehren M, Kane JM, Agulnik M, Bui MM, Ganjoo KN, George S, et al. Soft tissue sarcoma, version 2.2018, NCCN clinical practice guidelines in oncology. 2018;16(5):536-63.
42. Rueten-Budde AJ, van Praag VM, van de Sande MAJ, Fiocco M. Dynamic prediction of overall survival for patients with high-grade extremity soft tissue sarcoma. Surgical oncology. 2018;27(4):695-701.
43. Callegaro D, Miceli R, Bonvalot S, Ferguson PC, Strauss DC, van Praag VVM, et al. Development and external validation of a dynamic prognostic nomogram for primary extremity soft tissue sarcoma survivors. EClinicalMedicine. 2019;17:100215.
44. Kent DM, Paulus JK, van Klaveren D, D'Agostino R, Goodman S, Hayward R, et al. The Predictive Approaches to Treatment effect Heterogeneity (PATH) Statement. Ann Intern Med. 2020;172(1):35-45.
45. Cahlon O, Brennan MF, Jia X, Qin LX, Singer S, Alektiar KM. A postoperative nomogram for local recurrence risk in extremity soft tissue sarcomas after limb-sparing surgery without adjuvant radiation. Ann Surg. 2012;255(2):343-7.
46. Cahan EM, Hernandez-Boussard T, Thadaney-Israni S, Rubin DL. Putting the data before the algorithm in big data addressing personalized healthcare. NPJ Digit Med. 2019;2:78.
47. Char DS, Shah NH, Magnus D. Implementing Machine Learning in Health Care - Addressing Ethical Challenges. N Engl J Med. 2018;378(11):981-3.
48. Acem I, Smit MM, Verhoef C, van Houdt WJ, Haas RL, van der Hage JA, et al. Management of Soft Tissue Sarcomas in Extremities: Variation in Treatment Recommendations and Surveillance According to Specialty and Continent. Annals of surgical oncology. 2021;28(12):7923-36.
49. Kruiswijk AA, Kuhrij LS, Dorleijn DMJ, Van de Sande MAJ, van Bodegom-Vos L, Marang-van de Mheen PJ. Follow-Up after Curative Surgical Treatment of Soft-Tissue Sarcoma for Early Detection of Recurrence: Which Patients Have More or Fewer Visits than Advised in Guidelines? Cancers (Basel). 2023;15(18):4617.

50. Kruiswijk AA, Van Bodegom-Vos L. The Value of a Risk Prediction Tool (PERSARC) for Effective Treatment Decisions of Soft-tissue Sarcomas Patients 2021 [Available from: https://classic.clinicaltrials.gov/show/NCT05741944].
51. Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. Epidemiology. 2004;15(5):615-25.
52. Greenland S, Robins JM. Identifiability, exchangeability, and epidemiological confounding. International journal of epidemiology. 1986;15(3):413-9.
53. Chalmers TC, Celano P, Sacks HS, Smith H, Jr. Bias in treatment assignment in controlled clinical trials. N Engl J Med. 1983;309(22):1358-61.
54. Schulz KF, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. Jama. 1995;273(5):408-12.
55. Grimes DA, Schulz KF. Bias and causal associations in observational research. Lancet. 2002;359(9302):248-52.
56. Rubin DB. Estimating causal effects from large data sets using propensity scores. Ann Intern Med. 1997;127(8 Pt 2):757-63.
57. Baiocchi M, Cheng J, Small DS. Instrumental variable methods for causal inference. Stat Med. 2014;33(13):2297-340.
58. Desai RJ, Franklin JM. Alternative approaches for confounding adjustment in observational studies using weighting based on the propensity score: a primer for practitioners. Bmj. 2019;367:l5657.
59. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. Bmj. 2009;338:b2393.
60. White IR, Carlin JB. Bias and efficiency of multiple imputation compared with complete-case analysis for missing covariate values. Stat Med. 2010;29(28):2920-31.
61. Van Buuren S. Flexible Imputation of Missing Data. 2nd ed. New York: Chapman and Hall/CRC; 2018.

Chapter 13

Summary

Summary

This thesis aimed to contribute to a more patient-tailored approach in the management of soft tissue sarcomas (STS) and consists of three parts. In **part I** we explored differences in oncological outcomes in STS and malignant peripheral nerve sheath tumours (MPNST) and identified risk factors for poorer outcomes in the STS spectrum. In **part II** we developed and validated a diagnostic and prognostic tool in STS and MPNST, respectively. Finally, in **part III** we strived to gain more insight into the current management of STS and sought to improve the management of STS in a more patient-tailored manner using prediction tools.

PART I

In the first part of this thesis, titled ***'Risk factors and oncological outcome: heterogeneity within the sarcoma spectrum'***, we identified large variation in clinical presentation and oncological outcome in patients with STS. Some of this variation can be explained by differences in patient-specific characteristics, such as age (**chapter 2**). For example, young adults presented more often with 'whoops'-surgery, while older age was associated with poorer survival in patients with primary STS of the extremities (eSTS) (**chapter 2**). In addition, tumour-specific characteristics, including high grade, larger tumour size and certain genetic alterations, such as mutations in the NF1 and TP53 gene in MPNST, were associated with poorer oncological outcomes (**chapter 3, 4 and 5**).

PART II

In the second part of this thesis, ***'Development of prediction tools in STS: identifying patients at risk and predicting oncological outcome'***, we assessed the diagnostic ability of a new 'molecular fingerprint' based on exhaled breath in patients with STS. This pilot study demonstrated that exhaled breath could be a promising non-invasive diagnostic biomarker for the detection of STS with a good discriminative ability (C-statistic of 0.85) (**chapter 6**). Furthermore, we build a novel prognostic tool, the MPNST Oncological And Clinical Outcome Consortium (MONACO) prediction tool. This tool outperformed the existing generic STS prediction tools, Sarculator and PERSARC, by incorporating subtype-specific predictors for MPNST. (**chapter 7**).

PART III

In the final part of this thesis, ***'The management of soft tissue sarcoma: from one-size fits all to patient-tailored medicine'***, we aimed to provide an insight into clinical

decision-making of sarcoma specialists for patients with primary eSTS (**chapter 8**). This chapter demonstrated that specialty and continent are important factors contributing to the variation in clinical practice, treatment recommendations and follow-up in patients with primary eSTS. Furthermore, this study showed that the 62% of the respondents thought that there was insufficient evidence for the use of perioperative chemotherapy (CTX) in patients with primary high-grade eSTS. Nevertheless, 81% of the respondents would consider the use of a prediction tool as an instrument to assess which patients should get perioperative CTX recommended. Therefore, in **chapter 9** we aimed to assess the effect of perioperative CTX on overall survival and performed a risk-stratified analysis based on the PERSARC prediction tool, to identify which patients with high-grade eSTS might benefit from perioperative CTX. In this multicentre cohort study of more than 5500 patients, we found a beneficial effect of anthracycline and ifosfamide-based chemotherapy in a selected-group of high-risk patients with an absolute survival benefit of 11%. Moreover, we assessed the added value of restaging chest imaging after neoadjuvant radiotherapy (RTX) in patients with STS (**chapter 10**). This study demonstrated that routine chest imaging influenced the treatment strategy in 19% of the patients. This finding underlines the added value of routine restaging for distant metastasis with chest CT or X-ray after neoadjuvant RTX in patients with STS. Finally, in **chapter 11** we provided an overview of the available prediction tools for eSTS. Furthermore, we provided an approach for clinicians to evaluate the performance and usefulness of the available tools for their own patient population and discussed the possible applications of these tools in the clinical decision making and management of patients with STS.

Chapter 14

Nederlandse Samenvatting

Nederlandse samenvatting

Dit proefschrift had tot doel om bij te dragen aan een meer gepersonaliseerde en op de patiënt afgestemde benadering van weke delen sarcomen (STS). Dit proefschrift bestaat uit drie delen. In **deel I** hebben we gekeken naar verschillen in oncologische uitkomsten bij patiënten met STS en maligne perifere zenuwschede tumoren (MPNST) en identificeerden we risicofactoren voor slechtere uitkomsten bij STS. In **deel II** hebben we respectievelijk een diagnostisch en prognostisch instrument ontwikkeld en gevalideerd voor STS en MPNST. Ten slotte hebben we in **deel III** meer inzicht proberen te krijgen in het huidige behandeltraject van STS. Daarnaast hebben we gekeken naar hoe we het behandeltraject van STS kunnen verbeteren op een gepersonaliseerde manier met behulp van predictie instrumenten.

DEEL I

In het eerste deel van dit proefschrift, ***'Risicofactoren en oncologische uitkomsten: heterogeniteit binnen het sarcoomspectrum'***, hebben we grote verschillen in klinische presentatie en oncologische uitkomsten bij patiënten met STS geïdentificeerd. Sommige verschillen in klinische presentatie en oncologische uitkomsten kunnen worden verklaard door verschillen in patiënt-specifieke kenmerken, zoals leeftijd (**hoofdstuk 2**). Zo presenteren jongvolwassenen zich vaker met een 'whoops'-operatie en hadden oudere patiënten een slechtere overleving in vergelijking met jongere patiënten met primaire STS van de extremiteiten (eSTS) (**hoofdstuk 2**). Bovendien zijn tumor-specifieke kenmerken, zoals hoge graad, grotere tumoren en bepaalde genetische veranderingen, bijvoorbeeld mutaties in het NF1 en TP53-gen bij MPNST, geassocieerd met slechtere oncologische uitkomsten (**hoofdstuk 3, 4 en 5**).

DEEL II

In het tweede deel van dit proefschrift, ***'Ontwikkeling van predictie instrumenten voor STS: identificeren van risicopatiënten en voorspellen van oncologische uitkomsten'***, hebben we de diagnostische waarde van een nieuwe 'moleculaire vingerprint' op basis van uitgeademde lucht bij patiënten met STS onderzocht. Deze pilotstudie toont aan dat uitgeademde lucht een veelbelovende niet-invasieve diagnostische biomarker kan zijn voor de detectie van STS, met een goed onderscheidend vermogen (C-statistiek van 0,85) (**hoofdstuk 6**). Bovendien hebben we een nieuw prognostisch instrument ontwikkeld, het MPNST Oncological And Clinical Outcome Consortium (MONACO) predictie instrument. Dit instrument is het eerste predictie instrument met type-specifieke voorspellers voor MPNST en heeft

een beter voorspellend vermogen dan de bestaande generieke predictie instrumenten Sarculator en PERSARC (**hoofdstuk 7**).

DEEL III

In het laatste deel van dit proefschrift, ***'De behandeling van weke delen sarcoom: van one-size-fits-all naar gepersonaliseerde geneeskunde'***, streefden we ernaar om inzicht te bieden in de klinische besluitvorming van sarcoomspecialisten in eSTS (**hoofdstuk 8**). Dit hoofdstuk toont aan dat de variatie in klinische praktijk en behandeladviezen gerelateerd is aan het specialisme van de arts en het continent waar deze gevestigd is. Bovendien liet dit onderzoek zien dat 62% van de sarcoomspecialisten vindt dat er onvoldoende wetenschappelijk bewijs is voor het gebruik van perioperatieve chemotherapie (CTX) bij patiënten met primaire hooggradige eSTS. Toch zou 81% van de respondenten een predictie instrument willen gebruiken om te beoordelen welke patiënten perioperatieve CTX moeten krijgen. Daarom hebben we in **hoofdstuk 9** het effect van perioperatieve CTX op overleving onderzocht. We hebben een risico-gestratificeerde analyse uitgevoerd op basis van het PERSARC predictie instrument, om te identificeren welke groep aan patiënten met hooggradige eSTS baat heeft bij perioperatieve CTX. In deze multicenter cohortstudie met meer dan 5500 patiënten hebben we een gunstig effect van anthracycline- en ifosfamide-gebaseerde chemotherapie gevonden bij een selecte groep van hoog-risicopatiënten. Deze hoog-risicopatiënten hadden een overlevingswinst van 11% ten opzichte van hoog-risicopatiënten die geen perioperatieve chemotherapie hadden gekregen. In **hoofdstuk 10** onderzochten we wat de toegevoegde waarde is van routinematige herstageren middels longfoto's of longscans na neoadjuvante radiotherapie (RTX) bij patiënten met STS. Deze studie toonde aan dat routinematige herstageren de behandelstrategie beïnvloedde bij een aanzienlijk grote groep patiënten (19%). Deze bevinding benadrukt de toegevoegde waarde van routinematig herstagering voor DM met longfoto's of -scans in patiënten met STS na neoadjuvante RTX. Tot slot hebben we in **hoofdstuk 11** een overzicht gegeven van de beschikbare predictie instrumenten voor eSTS. Bovendien hebben we een benadering geboden voor clinici om de validiteit en bruikbaarheid van de beschikbare instrumenten voor hun eigen patiëntenpopulatie te evalueren. Tot slot, bespraken we de mogelijke toepassingen van deze predictie instrumenten in de klinische praktijk en hoe deze instrumenten een gepersonaliseerde benadering van patiënten met STS kan faciliteren.

APPENDICES

List of publications

In this thesis:

Noninvasive detection of soft tissue sarcoma using volatile organic compounds in exhaled breath: a pilot study.
Acem I, van Praag VM, Mostert CQ, van der Wal RJ, Neijenhuis RM, Verhoef C, Grünhagen DJ, van de Sande MA.
Future Oncol. 2023 Mar;19(10):697-704.

Prediction tools for the personalized management of soft-tissue sarcomas of the extremity.
Acem I, van de Sande MAJ.
Bone Joint J. 2022 Sep;104-B(9):1011-1016.

The added value of chest imaging after neoadjuvant radiotherapy for soft tissue sarcoma of the extremities and trunk wall: A retrospective cohort study.
Acem I, Schultze BTA, Schoonbeek A, van Houdt WJ, van de Sande MAJ, Visser JJ, Grünhagen DJ, Verhoef C.
Eur J Surg Oncol. 2022 Jul;48(7):1543-1549.

The role of perioperative chemotherapy in primary high-grade extremity soft tissue sarcoma: a risk-stratified analysis using PERSARC.
Acem I, van Houdt WJ, Grünhagen DJ, van der Graaf WTA, Rueten-Budde AJ, Gelderblom H, Verhoef C; PERSARC research group; van de Sande MAJ.
Eur J Cancer. 2022 Apr;165:71-80.

Management of Soft Tissue Sarcomas in Extremities: Variation in Treatment Recommendations and Surveillance According to Specialty and Continent.
Acem I, Smit MM, Verhoef C, van Houdt WJ, Haas RL, van der Hage JA, Grünhagen DJ, van de Sande MAJ.
Ann Surg Oncol. 2021 Nov;28(12):7923-7936.

The Association of Metastasis Pattern and Management of Metastatic Disease with Oncological Outcomes in Patients with Malignant Peripheral Nerve Sheath Tumors: A Multicenter Cohort Study.
Acem I, Martin E, van Houdt WJ, van de Sande MAJ, Grünhagen DJ, Verhoef C, Monaco Collaborators.
Cancers (Basel). 2021 Oct 12;13(20):5115.

Prognostic Significance of Immunohistochemical Markers and Genetic Alterations in Malignant Peripheral Nerve Sheath Tumors: A Systematic Review.
Martin E, Acem I, Grünhagen DJ, Bovée JVMG, Verhoef C.
Front Oncol. 2020 Dec 22;10:594069.

Age-related differences of oncological outcomes in primary extremity soft tissue sarcoma: a multistate model including 6260 patients.
Acem I, Verhoef C, Rueten-Budde AJ, Grünhagen DJ, van Houdt WJ, van de Sande MAJ; PERSARC study group.
Eur J Cancer. 2020 Dec;141:128-136.

Survival after resection of malignant peripheral nerve sheath tumours: Introducing and validating a novel type-specific prognostic model
Acem I, Steyerberg EW, Spreafico M, Grünhagen DJ, Callegaro D, Spinner RJ, Pendleton C, Coert JH, Miceli R, Abruzzese G, Flucke UE, Slooff WM, Van Dalen T, Been LB, Bonenkamp HJ, Anten MHME, Broen MPG, Bemelmans MHA, Bramer JAM, Schaap GR, Kievit AJ, Van der Hage J, Van Houdt WJ, Van de Sande MAJ, Gronchi A, Verhoef C, Martin E.
Submitted

Local recurrence in malignant peripheral nerve sheath tumours: A multicentre cohort study
Jansma CYMN, Acem I, Grünhagen DJ, Verhoef C, Martin E, and MONACO Collaborators
Submitted

Not in this thesis:

Effect of radiotherapy on local recurrence, distant metastasis and overall survival in 1200 extremity soft tissue sarcoma patients. Retrospective analysis using IPTW-adjusted models.
Smolle MA, Andreou D, Wölfel J, Acem I, Aj Van De Sande M, Jeys L, Bonenkamp H, Pollock R, Tunn PU, Haas R, Posch F, Van Ginkel RJ, Verhoef C, Liegl-Atzwanger B, Moustafa-Hubmer D, Jost PJ, Leithner A, Szkandera J.
Radiother Oncol. 2023 Dec;189:109944.

VISTA Expression on Cancer-Associated Endothelium Selectively Prevents T-cell Extravasation.
Luk SJ, Schoppmeyer R, Ijsselsteijn ME, Somarakis A, Acem I, Remst DFG, Cox DT, van Bergen CAM, Briaire-de Bruijn I, Grönloh MLB, van der Meer WJ, Hawinkels LJAC, Koning RI, Bos E, Bovée JVMG, de Miranda NFCC, Szuhai K, van Buul JD, Falkenburg JHF, Heemskerk MHM.
Cancer Immunol Res. 2023 Nov 1;11(11):1480-1492.

Intermuscular extremity myxoid liposarcoma can be managed by marginal resection following neoadjuvant radiotherapy.
Perera JR, AlFaraidy M, Ibe I, Aoude A, Acem I, van de Sande MAJ, Dessureault M, Turcotte RE, Mottard S, Basile G, Isler M, Saint-Yves H, Eastley N, Stevenson J, Houdek MT, Chung PWM, Griffin AM, Ferguson P, Wunder JS, Tsoi KM.
Eur J Surg Oncol. 2023 Feb;49(2):362-367.

Multimodality treatment of undifferentiated pleomorphic soft tissue sarcoma of the extremity (eUPS) in the elderly.
Bleckman RF, Acem I, van Praag VM, Dorleijn DMJ, Verhoef C, Schrage YM, Haas RML, van de Sande MAJ, The Collaborative Persarc Research Group; collaborative PERSARC research group.
Eur J Surg Oncol. 2022 May;48(5):985-993.

ASO Author Reflections: Towards Patient-Tailored Management of Extremity Soft Tissue Sarcoma.
Acem I, van de Sande MAJ, Verhoef C.
Ann Surg Oncol. 2021 Nov;28(12):7937-7938.

Survival Analysis of 3 Different Age Groups and Prognostic Factors among 402 Patients with Skeletal High-Grade Osteosarcoma. Real World Data from a Single Tertiary Sarcoma Center.
Evenhuis RE, Acem I, Rueten-Budde AJ, Karis DSA, Fiocco M, Dorleijn DMJ, Speetjens FM, Anninga J, Gelderblom H, van de Sande MAJ.
Cancers (Basel). 2021 Jan 27;13(3):486.

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PHD PORTFOLIO

Year		ECTS
	Courses	
2022	An Introduction to the Analysis of the Next-Generation Sequencing Data (MEP-EL019)	1.4
2021	eBROK	1.5
	Scientific Integrity	0.3
2020	Pharmaco-epidemiology and Drug Safety (EWP03)	1.9
	Advanced Analysis of Prognosis Studies (EWP13)	0.9
	Advanced Clinical Trials (EWP10)	1.9
	Scientific writing	2.0
	Competing Risks and Multi-state Models (BST03)	0.9
	Review of Mathematics and Introduction to Statistics (BST01)	1.4
2019	Missing Values in Clinical Research (EP16)	1.7
	Repeated Measurements (CE08)	1.7
	Intermediate course in R (BST02)	1.4
	Using R for Decision Modelling, Simulation, and Health Technology (CE16)	1.1
	Advanced Topics in Decision Making in Medicine (EWP02)	2.4
	Advanced Decision Modelling (CE15)	1.4
	Biostatistical Methods II: Classical Regression Models (EP03)	4.3
2018	Principles in Causal Inference (EP01)	1.4
	Clinical Epidemiology (CE02)	3.7
	Clinical Translation of Epidemiology (CE01)	2.0
	Biostatistical Methods I: Basic Principles (CC02)	5.7
	Study Design (CC01)	4.3
	Value Based Healthcare, from theory to implementation (ESP76)	0.7
	The Practice of Epidemiologic Analysis (ESP65)	0.7
	Fundamentals of Medical Decision Making (ESP70)	0.7
	Methods of Public Health Research (ESP11)	0.7
	Clinical Trials (ESP14)	0.7
	Principles of Research in Medicine and Epidemiology (ESP01)	0.7
	Oral Presentations	
2022	EMSOS congress, London/virtual	0.5
	ISOLS congress, Los Angeles/virtual	0.5
	DSG symposium, Utrecht	0.5
2021	EMSOS congress, virtual [2x]	1.0
	CTOS congress, virtual [2x]	1.0
	BOOS congress, London/virtual	0.5
	DSG symposium, virtual	0.5
	Poster presentations	
2022	CTOS congress	0.3

2021	ESSO congress, Lisbon [3x]	0.9
2020	CTOS congress, virtual	0.3
	(Inter)national conferences and symposia	
2022	EMSOS congress, London	0.9
	DSG symposium, Utrecht	0.3
	NOV jaarcongres, Utrecht	0.3
	Sarcoma Academy Symposium 'Predictive measures for individualizing therapy incl. clinical trials', virtual	0.1
2021	EMSOS congress, virtual	0.9
	CTOS congress, virtual	1.2
	BOOS congress, London/virtual	0.3
	NOV jaarcongres, 's-Hertogenbosch	0.3
	DSG symposium, virtual	0.3
2020	CTOS congress, virtual	1.2
	Meetings	
2020-2022	Surgical Journal Club of Clinical Oncology (SJOCO), Rotterdam	0.5
	Research meetings 'Oncologische Gastro-intestinale Chirurgie' (OCG), Rotterdam	0.5
	Research meetings dept. Orthopaedic surgery, Leiden	1.0
	Journal clubs dept. Orthopaedic surgery and Medical Decision Making	0.5
	Other	
2020-2022	Erasmusarts 2030, Werkgroep Academische Vorming	2
2021	Moderator Flash Session STS, EMSOS congress, virtual	0.3
	Teaching activities	
2021	Supervising master thesis, Erasmus MC, B.T.A. Schultze	1.5
	Supervising bachelor thesis, LUMC, L. Hoeve	0.5
2020	Supervising master thesis, LUMC, M.M.J. Smit	1.5
	Supervising master student, LUMC, R.F. Bleckman	0.5
Total		**68.1**

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About the author

Ibtissam Acem was born on December 31st, 1996, in Woerden, the Netherlands. She completed secondary school at the Minkema College (Gymnasium) in Woerden in 2015 and commenced her bachelor's degree in Medicine at Erasmus University Rotterdam. During her undergraduate years, Ibtissam served as a board member of De Geneeskundestudent, a national advocacy organization for medical students affiliated with the Royal Dutch Medical Association (KNMG). Throughout her tenure on the board, she wrote several reports on career choices and the medical labor market, and actively participated in various working groups with, among others, the Ministry of Social Affairs and the Ministry of Education, Culture, and Science.

Upon earning her bachelor's degree in 2018, Ibtissam embarked on a two-year research master's program in Clinical Research at the Netherlands Institute of Health Sciences (NIHES), concurrently pursuing a master's degree in Health Economics, Policy, and Law at the Erasmus School of Health Policy & Management (ESHPM). Her interest beyond medicine led her to further (pre)master studies in Health Law at the Erasmus School of Law (ESL).

Following the completion of her research master's in Clinical Research in 2020, she continued her research projects as a PhD student at the department of Surgical Oncology at the Erasmus MC Cancer Institute in Rotterdam, under the supervision of Prof. Dr. C. Verhoef, Prof. Dr. M.A.J. van de Sande, Dr. D.J. Grünhagen, and Dr. W.J. van Houdt. During these years, Ibtissam has put her interest in education and advocacy into practice by starting a podcast series on career orientation (MATCH podcast) and joining the members' council of the National Association of Salaried Doctors (LAD) representing medical interns. Additionally, she joined the working group 'Academic Formation' of Erasmusarts 2030, developing academic education for future medical students at the Erasmus MC.

In November 2022, she pursued her master's degree in Medicine at Erasmus University Rotterdam. Currently, Ibtissam is in the final stages of her clinical rotations and her master's in Health Law. After finishing her medical degree, she plans to persue her career as a medical doctor and clinical epidemiologist, aiming to bridge the gap between research and clinical practice.