Tumour Marker Prognostic Study

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Plasma YKL-40 as a biomarker in patients with nonmetastatic bone and soft tissue sarcomas: a prospective exploratory clinical study

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Purpose: YKL-40 is a glycoprotein with a role in inflammation, tissue remodeling, tumor angiogenesis, and protection against apoptosis. We hypothesized that high preoperative plasma YKL-40 in patients with nonmetastatic bone and soft tissue sarcoma (STS) is associated with short overall survival (OS), and that plasma YKL-40 is an independent predictor for OS.

Materials and methods: Plasma was collected preoperatively from 65 patients with nonmetastatic bone (n = 14) or STS (n = 51) in the lower extremities (n = 43), the upper extremities (n = 16) or the trunk wall/spine (n = 6). All patients underwent surgical cancer treatment. Twenty patients developed metastases during the follow-up period (minimum 5 y). The plasma concentration of YKL-40 was determined by enzyme-linked immunosorbent assay.

Results: Twenty-seven patients died [mean: 3.2 (range: 0.2–7.3) y postoperatively] and 38 patients were still alive after a follow-up of mean 6.9 (5.8–8.2) years postoperatively. Plasma YKL-40 was higher in patients who died during follow-up ($P = 0.008$), in males ($P = 0.007$) and in patients 61 years of age and above ($P = 0.001$). The 5-year OS was 68% and OS was lower in patients with high ($\geq$95% percentile age-corrected) plasma YKL-40 ($P = 0.021$), age 61 years and above ($P = 0.013$), high histologic malignancy grade ($P = 0.047$) and male sex ($P = 0.051$). Multivariable analysis showed that only plasma YKL-40 (age-corrected (hazard ratio = 2.80, 95% confidence interval: 1.13–6.91, $P = 0.026$) and malignancy grade (hazard ratio = 9.9×10^7, 95% confidence interval: 0–∞, $P = 0.007$) remained independent prognostic factors for OS.

Conclusions: High preoperative plasma YKL-40 was related to short OS in patients with nonmetastatic bone and STS and plasma YKL-40 (age-corrected) was an independent prognostic risk factor for OS.

Keywords: YKL-40, Prognosis, Survival, Nonmetastatic bone sarcoma, Nonmetastatic soft tissue sarcomas

Background

YKL-40 (also named chitinase 3 like protein 1) is a 40 KDa glycoprotein, and it was first described in 1992, where it was found to be secreted in vitro by the human osteosarcoma cell line MG-63[1]. Later studies demonstrated YKL-40 expression in soft tissue sarcomas (STS), chondrosarcomas, and osteosarcomas[2], and that high YKL-40 expression was related to short survival in STS[3]. It has also been found that YKL-40 expression is related to malignancy grade in STS, but not in chondrosarcomas[3,4]. Furthermore, YKL-40 is a growth factor for fibroblasts, chondrocytes, bone cells, and connective tissue[5–8].

YKL-40, which is encoded by the *CHI3L1* gene, is produced by tumor cells[9,10], inflammatory cells[11,12], and stem cells[13]. YKL-40 interacts with the receptors IL-13Rα2[14], CRTH2, RAGE[15], syndecan-1[6], and PAR-2[16], and a physiological ligand for YKL-40 is hyaluronan[17]. YKL-40 plays important functions in angiogenesis, inflammation[11,12,18,19], fibrosis[20,21], tissue remodeling, protection against apoptosis, and has also shown to be a growth factor for fibroblasts cartilage, bone cells, and connective tissue[5–8]. Furthermore, accumulating evidence has shown high circulating levels of YKL-40 in patients with various types of cancer (breast, colorectal, bladder, ovarian, kidney, prostate, malignant melanoma, and small cell lung cancer), and that serum/plasma levels of YKL-40 can be used as an independent prognostic marker for overall short-term survival[22,23]. YKL-40 is, however, not specific for cancer, since high serum/plasma levels have also been found in many non-malignant inflammatory diseases such as pneumonia, osteoarthritis, rheumatoid arthritis, and inflammatory bowel disease[4,24,25].

Objectives

In this prospective biomarker study, we examined the prognostic value of preoperative plasma YKL-40 in patients with nonmetastatic

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bone and STS. We hypothesized that high preoperative plasma YKL-40 in these patients is associated with short overall survival (OS), and that plasma YKL-40 is an independent predictor for OS.

Material and methods

Patients

Sixty-five patients operated on for the first time for a biopsy verified STS (n = 51) or bone sarcoma (n = 14) at the Musculoskeletal Tumor Section, Rigshospitalet, University of Copenhagen were included in the study. The patients were included during the period December 2009 to April 2012, and in that period the department operated ~60–80 newly diagnosed sarcoma cases each year. Seven patients initially included in the study were excluded because the diagnosis was later changed to malignant melanoma metastasis (n = 1), lipoma (n = 1), aneurysmal bone cyst (n = 1), and atypical lipomatous tumors (n = 4). One patient was excluded due to metastases present at the time of diagnosis. The number of patients included in the present study was therefore 65. The clinical characteristics of patients and tumor characteristics are summarized in **Table 1**.

From the electronic patient files information regarding metastasis status at the time of diagnosis or later, chemotherapy treatment, surgical margin, local recurrence, tumor type, and subtype were collected and updated by January 1, 2018. This resulted in a minimum follow-up period of 5 years for patients still alive at the time of follow-up, and a maximum follow-up of 8 years (range: 5.8–8.2 y). Data from The Danish Civil Registration system[26] was used for information regarding vital status, thus no patients were lost to follow-up for survival status.

The majority of tumors were located at the lower extremities (n = 43) or upper extremities (n = 16), and a small number was located in the trunk wall or spine (n = 6). Using the FNCLCC (French Fédération Nationale des Centres de Lutte Contre le Cancer) Trojani grading system[27] the tumors were classified as low malignant [Trojani grade I (n = 6)], or high malignant (n = 46) [Trojani grade II (n = 23) or III (n = 23)]. A total of 22 different histologic types of sarcomas were included in the study, and the most common types were myxofibrosarcomas (n = 11), sarcomas not otherwise specified (n = 9), and chondrosarcomas (n = 8) (**Table 1**).

All 65 patients underwent surgical treatment for their cancer. In 34 patients, a wide margin was obtained, while 23 patients had the tumor removed with a marginal margin. In 3 patients, the tumor was removed with a positive margin and in 5 patients the surgical margin was not described. Thirteen patients received chemotherapy [postsurgery (n = 10), presurgery and postsurgery (n = 3)], and 27 patients were treated with radiotherapy [postsurgery (n = 20), perioperative brachytherapy combined with later external radiation therapy (n = 2), perioperative brachytherapy alone (n = 3), and as a part of palliative treatment (n = 2) because of a local recurrence during the follow-up period].

Table 1

Baseline characteristics for all patients.

Parameters	n
Sex	
Male	32
Female	33
Age (y)	
Mean (range) age (y)	61 (29–90)
Site	
Trunk wall/spine	6
Upper extremity	16
Lower extremity	43
Patients with metastases during follow-up	20
Location of postoperative metastases	
Lung	15
Bone	1
Abdomen	1
Lever	1
Muscle	2
Tumor size (cm)	
≤ 8	27
> 8	30
Alive at follow-up	38
Malignancy grade	
Low: Trojani I	6
High: Trojani II	23
High: Trojani III	23
Local recurrence	11
Tumor type	
Bone sarcoma	14
Soft tissue sarcoma	51
Tumor subtype	
Adamantinoma	1
Chordoma	2
Chondroblastic osteosarcoma	1
Chondrosarcoma	8
Dedifferentiated chondrosarcoma	1
Dedifferentiated liposarcoma	1
Dermatofibrosarcoma protuberans	1
Epithelioid sarcoma	1
Ewing sarcoma	1
Fibrosarcoma	1
Leiomyosarcoma	2
Monophasic synovial sarcoma	2
Myofibrosarcoma	2
Myxofibrosarcoma	11
Myxoid liposarcoma	7
Osteosarcoma	2
Pleomorphic liposarcoma	2
Pleomorphic Rhabdomyosarcoma	1
Pleomorphic undifferentiated sarcoma	5
Sarcoma NOS	9
Subcutaneous sarcoma	1
Synovial sarcoma	3

NOS indicates not otherwise specified.

Plasma YKL-40 analysis

The blood sample was taken before a patient's first surgery either the day before surgery or on the day of surgery. Blood was drawn into a 6 mL ethylene-diamine-tetra-acetic acid (EDTA) tube and stored between 1/2 –2 hours at room temperature, and then centrifuged for 10 minutes at 3000 rounds per minute. Thereafter plasma was isolated and then stored at –80°C. YKL-40 was determined in duplicate in the preoperative plasma samples using an enzyme-linked immunosorbent assay (ELISA) (Quidel Corporation, San Diego, CA) according to the manufacturer's instructions. The marker assessments were made blinded to clinical outcome. YKL-40 ELISA characteristics were: detection limit 20 ng/mL and intra-assay and interassay coefficients of variation (CVs) <5% and <6%[28]. An age-corrected 95th percentile for YKL-40 in healthy individuals served as a cut-off for elevated values and was calculated using the formula proposed by

Bojesen et al[28]:

$$\text{Percentile} = \frac{100}{1 + \left(\text{YKL-}40^{-3}\right) \times \left(1.062^{\text{age}}\right) \times 5000}.$$

Subgroup analysis

In order to analyze the relation of preoperative plasma YKL-40 to various different demographic, clinical and histologic parameters, the patients were divided into the following subgroups: (1) sex: male/female (n = 32/n = 33); (2) mean age: 61 years and younger/61 years and above (n = 28/n = 37); (3) sarcoma type: bone/soft tissue: (n = 14/n = 51); (4) malignancy grade: low grade (Trojani I)/high grade Trojani II + III (n = 6/n = 46). Thirteen patients did not have data on malignancy grade available; (5) tumor size: ≤ 8 cm/ > 8 cm (n = 27/n = 30). Tumor size information was not available from 6 patients. Tumor size is a well-known parameter of importance for survival and we selected 8 cm according to the system for prognosis of patients with STS called the SIN system (tumor size, vascular invasion and microscopic tumor necrosis)[29]; (6) alive at follow-up: yes/no (n = 38/n = 27); (7) local recurrence: yes/no (n = 11/n = 54); (8) metastases during follow-up: yes/no (n = 20/n = 45); and (9) plasma YKL-40: <95 percentile/ ≥ 95 percentile of healthy subjects (n = 53/n = 12).

Statistics

Statistical analyses were performed using SPSS version 22 for Mac and the software R (R Foundation, Vienna, Austria). *P*-values <0.05 were considered statistically significant, and results are given as a median or mean and total range (if no other information is provided). Nonparametric test for unpaired data (Mann-Whitney) was used to determine potential differences between plasma YKL-40 concentrations in various subgroups. Kaplan-Meier survival analysis was used to determine the probability of 5- and 8-year OS, and a 95% confidence interval (95% CI) for OS. The log-rank test was performed to compare the OS between subgroups. To make allowance for death as a competing risk factor, the cumulated incidence of local recurrence was calculated by Aalen-Johansen's analysis with death as a competing risk for local recurrence, and the difference in risk between subgroups was assessed by the Gray test. Finally, Cox-regression analysis (univariate/multivariable) with the calculation of hazard ratio (HR) with 95% CI was performed to identify possible independent prognostic factors available at the time of operation.

Ethics and study registration

The study was approved by the Scientific Ethics Committee of the Capital Region of Denmark (HD-2007-075) and is a part of a larger research project approved by the Danish Data Protection Agency (2007-41-1506). The study was registered on: https://www.researchregistry.com/ (Research Registry UIN: researchregistry5419).

The study was conducted in accordance with the Helsinki declaration with informed consent obtained (after written and oral information) from all study participants prior to inclusion in the study.

Reporting recommendations for tumor marker prognostic studies (REMARK)

The work in this study have been reported in line the with the REMARK[30].

Results

Plasma YKL-40 levels in sarcoma patients

The median preoperative plasma YKL-40 concentration in the patients with nonmetastatic bone and STS was 75 μg/L (18–1056 μg/L). Fifty-three (82%) patients [mean age (range) = 59 (29–85), F/M = 27/26, bone sarcomas/STS = 12/41] had normal plasma YKL-40 (ie, below the 95% age-corrected percentile) and 12 (19%) patients [mean age (range) = 69 (32–90), F/M = 6/6, bone sarcomas/STS = 2/10] had elevated plasma YKL-40 (ie, above the 95% age-corrected percentile) (**Table 2**). Males had a higher median plasma YKL-40 (99 μg/L) compared with women (68 μg/L) ($P = 0.007$), and patients older than 61 years had higher median plasma YKL-40 (97 μg/L) compared with the younger patients (64 μg/L) ($P = 0.001$) (**Table 2**). No statistically significant difference was found in median plasma YKL-40 levels when the patients were divided into the subgroups based upon

Table 2

Preoperative plasma YKL-40 levels according to various clinical characteristics.

Characteristics	Number	Plasma YKL-40 μg/L, Median (Range)	*P*
No. patients	65	75 (18–1056)	
Normal or elevated YKL-40			
< 95 age-corrected percentile	53	68 (18–186)	< 0.0005
≥ 95 age-corrected percentile	12	396 (77–1056)	
Sex			
Female	33	68 (18–858)	0.007
Male	32	99 (37–1056)	
Mean age (y)			
≤ 61	28	64 (18–570)	0.001
> 61	37	97 (20–1056)	
Sarcoma type			0.18
Bone sarcoma	14	97 (43–507)	
Soft tissue sarcoma	51	71 (18–1056)	
Malignancy grade			
Low (Trojani I)	6	58 (18–210)	0.078
High Trojani II + III	46	79 (25–1056)	
Tumor size (cm)			
≤ 8	28	68 (18–210)	0.175
> 8	29	77 (18–1056)	
Alive at follow-up			
Yes	38	69 (18–858)	0.008
No	27	94 (31–1056)	
Local recurrence during follow-up			
Yes	11	146 (18–1056)	0.09
No	54	73 (31–858)	
Metastases during follow-up			
Yes	20	79 (31–1056)	0.88
No	45	75 (18–858)	

Median (range) plasma YKL-40 levels in various subgroups. Differences between subgroups are tested using Mann-Whitney test.

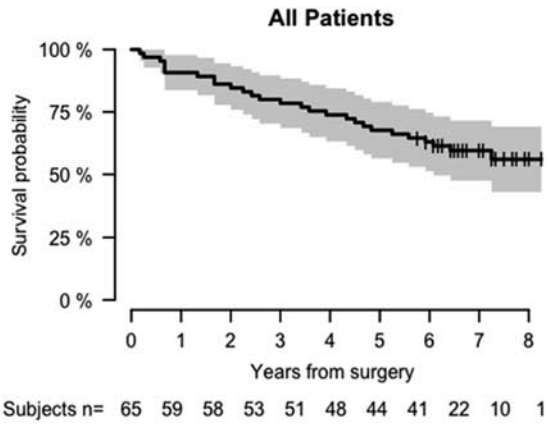

Figure 1. Kaplan-Meier survival analysis (with 95% confidence interval) of 65 patients with nonmetastatic bone (n = 14) and soft tissue sarcoma (n = 51).

sarcoma type (bone/soft tissue), malignancy grade, tumor size (≤ 8 cm/> 8 cm), local recurrence during the follow-up (yes/no) or the occurrence of distant metastases during follow-up (yes/no). However, a tendency to higher median plasma YKL-40 was seen in patients with high grade tumors ($P = 0.078$), and in patients that experienced a local recurrence ($P = 0.09$) (**Table 2**).

OS and local recurrence after operation

A total of 27 (42%) patients died during the follow-up period of mean 3.2 (0.2–7.3) years postoperatively. Thirty-eight (58%) patients were still alive after a follow-up of mean 6.9 (5.8–8.2) years postoperatively. Preoperative median plasma YKL-40 was higher in the patients who died during the follow-up period [94 μg/L (31–1056)] compared with the patients still alive [69 μg/L (18–858) ($P = 0.008$) (**Table 2**). Kaplan-Meier survival analysis showed that the probability of 5- and 8-year survival of all 65 patients were 68% (95% CI: 56%–79%) and 56% (95% CI: 43%–69%), respectively (**Fig. 1**).

Among the preoperative available parameters used for division into subgroups, log-rank test showed a statistically significant (or marginally significant) influence on OS for age-corrected plasma YKL-40 ($P = 0.021$), age ($P = 0.013$), malignancy grade ($P = 0.047$), and sex ($P = 0.051$), while no influence on OS was found for tumor size ($P = 0.45$) or sarcoma type ($P = 0.34$) (**Fig. 2**). OS was lower in patients that developed metastases during follow-up after surgery ($P < 0.001$), but also in patients that experienced a local recurrence, a tendency to lower survival was found ($P = 0.069$) (**Fig. 2**).

Eleven patients experienced a local recurrence within the first 5 years postoperatively and the cumulated incidence of local recurrence (calculated by a competing risk regression model with death as a competing risk) was 7.7% (95% CI: 1.2%–14%) and 17% (95% CI: 7.8%–26%) after, respectively, 1 and 5 years of follow-up (**Fig. 3**). Age-corrected plasma YKL-40 was not a statistically significant factor for development of local recurrence ($P = 0.325$) (**Fig. 3**).

Prognostic preoperative variables for OS in sarcoma patients

A Cox regression analysis including all available preoperative parameters showed in the univariate analysis that the age-corrected plasma YKL-40 (HR = 2.56, 95% CI: 1.12–5.87, $P = 0.039$), age (HR = 2.86, 95% CI: 1.21–6.78, $P = 0.011$), malignancy grade (HR = 8.5×10^7, 95% CI: 0–∞, $P = 0.007$), and sex (HR = 0.47, 95% CI: 0.21–1.02, $P = 0.051$) were prognostic factors for OS, while tumor size ($P = 0.46$) and sarcoma type ($P = 0.31$) were not (**Table 3**). When entering all 4 prognostic factors into a multivariable analysis only the age-corrected plasma YKL-40 (HR = 2.80, 95% CI: 1.13–6.91, $P = 0.026$) and malignancy grade (HR = 9.9×10^7, 95% CI: 0–∞, $P < 0.001$) remained independent prognostic factors for OS. Sex (HR = 0.50, 95% CI: 0.21–1.18, $P = 0.112$) and age (HR = 1.38, 95% CI: 0.55–3.45, $P = 0.493$) were not independent prognostic factors for OS (**Table 3**).

Discussion

We found that the age-corrected plasma YKL-40 measured at the time of surgery and malignancy grade are independent prognostic risk factors for OS in patients operated on for nonmetastatic bone and STS. The influence of plasma YKL-40 is a new observation, but the result is in good agreement with another study investigating the prognostic value of YKL-40 protein expression in sarcoma tissue[3]. The influence of the histologic malignancy grade on patient survival has previously been established, and our results confirm this well-known prognostic effect of this parameter[27,29].

The exact function of YKL-40 in nonmetastatic bone and STS is unknown. Many studies have focused on the function of YKL-40 in other cancer types and have found that neutrophils and tumor-associated macrophages in tumor cells or their surrounding microenvironment can secrete YKL-40 into the extracellular space. Here YKL-40 can induce tumor growth via cancer cell proliferation, inhibition of epithelial cell apoptosis, and promotion of angiogenesis. Furthermore, YKL-40 can increase the invasion of cancer cells and thereby induce tumor progression and metastasis[9]. YKL-40 is not cancer specific, and plasma YKL-40 is elevated in patients with diseases characterized by inflammation such as rheumatoid arthritis, bacterial infection, lung diseases, and inflammatory bowel disease, and elevated plasma YKL-40 is associated with more active disease[10,12,31].

The inflammatory process around the tumor is critical for tumor progression in many tumors, and this process is also upregulated by YKL-40[19]. This raises the question, how much plasma YKL-40 is due to underlying inflammatory disease in the body, and how much is a reflection of the tumor cells, their surrounding microenvironment, inflammatory response, and the malignity level of the tumor. Further studies are needed to evaluate this, perhaps studies that compare YKL-40 expression in tumor tissue and the circulating plasma YKL-40 in the same cancer patients.

We found lower plasma YKL-40 concentrations in patients under the age of 61 years, and lower levels in females compared with males. Age and sex are well-known prognostic markers in sarcoma patients and confirmed in studies with age cut-offs ranging from above 45 to above 64 years[32–35]. Circulating YKL-40 increases with age in healthy subjects. Bojesen et al[28] described an age-adjusted reference level for plasma YKL-40, and proposed that elevated plasma YKL-40 levels should be defined as higher than the 95th or the 97.5th percentile. In the present study, we defined an elevated preoperative plasma YKL-40 level as higher than the age-adjusted 95% percentile[28] and found that

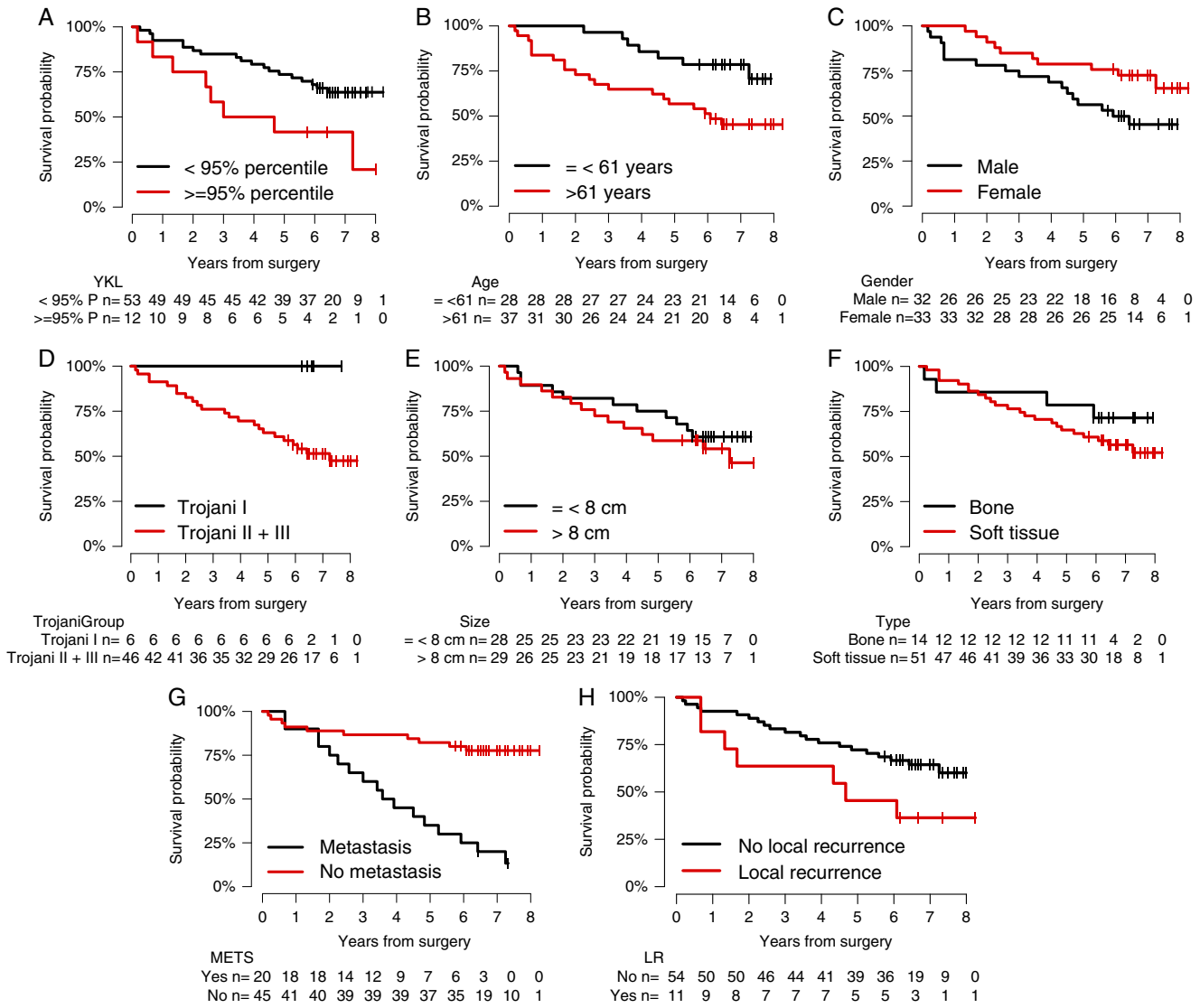

Figure 2. Kaplan-Meier survival analysis (with 95% confidence interval and log-rank test) comparing overall survival in various subgroups: (A) age-corrected plasma YKL-40 ($P = 0.021$); (B) age ($P = 0.013$); (C) sex ($P = 0.051$); (D) histologic malignancy grade ($P = 0.047$); (E) tumor size ($P = 0.45$); (F) sarcoma type ($P = 0.34$); (G) development of metastasis after operation ($P < .001$); and (H) local recurrence after operation ($P = 0.069$).

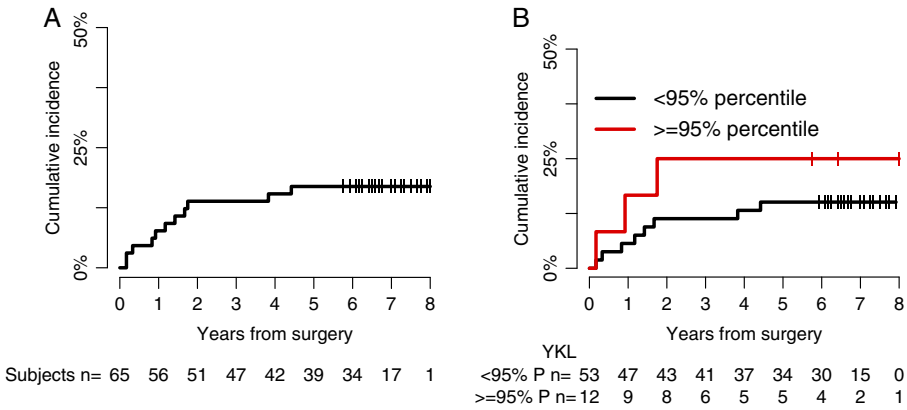

Figure 3. Cumulative incidence (with 95% confidence interval) for analysis of the risk of local recurrence in all patients (A), and the difference in risk between 2 subgroups with either normal (< 95 percentile) or increased (≥ 95 percentile) age-corrected plasma YKL-40 concentration (assessed by Gray test ($P = 0.325$) (B).

Table 3

Cox-regression analysis (univariate/multivariable) of possible prognostic factors at the time of operation in 65 bone and soft tissue sarcomas.

		Univariate			Multivariable		
Factor	**n**	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
YKL-40 percentile							
< 95	53	Ref.			Ref.		
≥ 95	12	2.56	1.12–5.87	0.039	2.80	1.13–6.91	0.026
Sex							
Male	32	Ref.			Ref.		
Female	33	0.47	0.21–1.02	0.051	0.50	0.21–1.18	0.112
Age (y)							
≤ 61	28	Ref.			Ref.		
> 61	37	2.86	1.21–6.78	0.011	1.38	0.55–3.45	0.493
Malignancy grade (n = 52)							
Low	6	Ref.			Ref.		
High	46	8.5×10^7	0–∞	0.007	9.9×10^7	0–∞	< 0.001
Sarcoma type							
Bone	14	Ref.					
Soft	51	1.66	0.58–4.84	0.313			
Tumor size (cm) (n = 57)							
≤ 8	28	Ref.					
> 8	29	1.35	0.61–2.98	0.455			

CI indicates confidence interval; HR, hazard ratio; Ref., reference.

this cut-off was useful to identify a subgroup of sarcoma patients with at very poor prognosis after tumor resection.

In general, the 5-year OS in patients with STS is ~49%–76% and for bone sarcoma 48%–69%[33,36–40]. We found a relatively high 5-year OS of 67% of the patients included in the present study. This could be explained by the fact that the patients included in the present study were all nonmetastatic at the time of surgery, and only represent a part of the subgroup of nonmetastatic bone and STS operated at our orthopedic oncology unit.

We included patients with 22 different histologic types of nonmetastatic bone and STS but did not have patients with all types of sarcomas. There were too few patients of each sarcoma type to make a comparison regarding plasma YKL-40 levels according to sarcoma type. Thus, future larger studies focusing on plasma YKL-40 and different histologic types of sarcomas are needed to determine, if plasma YKL-40 is a type-specific prognostic biomarker in patients with sarcomas.

YKL-40 is not specific for cancer, and we found no difference in plasma YKL-40 between patients who developed metastases during follow-up, and those that did not, thus indicating that comorbidity could also be of importance. We consider it a limitation of our study that comorbidity was not evaluated. We found that plasma YKL-40 was an independent prognostic factor for survival in patients with sarcoma, but this result should be interpreted with caution as the regression analysis does not oblige to the rule of 10 events per included variable[41]. It has been suggested that this rule is very conservative[42], and our analysis is not overfitted as the CI and the odds ratio does not change drastically between univariate and multivariable analysis. Thus, we do not think that we have violated the assumption for multivariable analysis. As we did not include a validation cohort, we underline that our findings should be interpreted more as a hypothesis-generating and not definitive, as we have not validated our findings in an independent cohort.

Conclusions

We found the following new observations not previously published:

- High preoperative plasma YKL-40 is related to short OS in patients with nonmetastatic bone and STS.
- Age-corrected plasma YKL-40 level measured preoperatively is an independent prognostic risk factor for OS in patients with nonmetastatic bone and STS.

Plasma YKL-40 is probably not sarcoma specific, but more likely a general prognostic factor for survival and further studies also including information on comorbidity in larger study populations are warranted.

Ethical approval

The study was approved by the Scientific Ethics Committee of the Capital Region of Denmark (HD-2007-075) and is a part of a larger research project approved by the Danish Data Protection Agency (2007-41-1506).

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Author contribution

A.P.T.: collected, analyzed and interpreted most of the data and was a major contributor in writing of the manuscript. M.L.H.: was part of the acquisition of the data. G.S.L.: made a substantial contribution to the concept of the study. J.S.J.: has been a major part of analysis of the data and revision of the article. M.S.S.: made a substantial contribution to the analysis of data, specifically statistics and in the revision of the article. M.M.P.: has been a substantial part of the concept of the study, analyze and interpreting of the data and in drafting and revision of the manuscript. All authors have read and approved the final manuscript.

Conflict of interest disclosure

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Research registration unique identifying number (UIN)

The study was registered on: https://www.researchregistry.com/ (Research Registry UIN: researchregistry5419).

Guarantor

Not applicable.

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References

[1] Johansen JS, Williamson MK, Rice JS, *et al.* Identification of proteins secreted by human osteoblastic cells in culture. J Bone Miner Res 1992;7:501–12.

[2] Thorn AP, Daugaard S, Christensen LH, *et al.* YKL-40 protein in osteosarcoma tumor tissue. APMIS 2016;124:453–61.

[3] Harving ML, Christensen LH, Ringsholt M, *et al.* YKL-40 expression in soft-tissue sarcomas and atypical lipomatous tumors. An immunohistochemical study of 49 tumors. Acta Orthop 2014;85:195–200.

[4] Daugaard S, Christensen LH, Hogdall E. Markers aiding the diagnosis of chondroid tumors: an immunohistochemical study including osteonectin, bcl-2, cox-2, actin, calponin, D2-40 (podoplanin), mdm-2, CD117 (c-kit), and YKL-40. APMIS 2009;117:518–25.

[5] Johansen JS, Hoyer PE, Larsen LA, *et al.* YKL-40 protein expression in the early developing human musculoskeletal system. J Histochem Cytochem 2007; 55:1213–28.

[6] Shao R, Hamel K, Petersen L, *et al.* YKL-40, a secreted glycoprotein, promotes tumor angiogenesis. Oncogene 2009;28:4456–68.

[7] Liu X, Zhang Y, Zhu Z, *et al.* Elevated pretreatment serum concentration of YKL-40: an independent prognostic biomarker for poor survival in patients with colorectal cancer. Med Oncol 2014;31:85.

[8] Johansen JS, Christensen IJ, Riisbro R, *et al.* High serum YKL-40 levels in patients with primary breast cancer is related to short recurrence free survival. Breast Cancer Res Treat 2003;80:15–21.

[9] Kzhyshkowska J, Yin S, Liu T, *et al.* Role of chitinase-like proteins in cancer. Biol Chem 2016;397:231–47.

[10] Schultz NA, Johansen JS. YKL-40-A protein in the field of translational medicine: a role as a biomarker in cancer patients? Cancers 2010;2: 1453–91.

[11] Hamilton G, Rath B. Circulating tumor cell interactions with macrophages: implications for biology and treatment. Transl Lung Cancer Res 2017;6:418–30.

[12] Lee CG, Da Silva CA, Dela Cruz CS, *et al.* Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Ann Rev Physiol 2011;73:479–501.

[13] Brochner CB, Johansen JS, Larsen LA, *et al.* YKL-40 is differentially expressed in human embryonic stem cells and in cell progeny of the three germ layers. J Histochem Cytochem 2012;60:188–204.

[14] He CH, Lee CG, Dela Cruz CS, *et al.* Chitinase 3-like 1 regulates cellular and tissue responses via IL-13 receptor alpha2. Cell Rep 2013;4:830–41.

[15] Low D, Subramaniam R, Lin L, *et al.* Chitinase 3-like 1 induces survival and proliferation of intestinal epithelial cells during chronic inflammation and colitis-associated cancer by regulating S100A9. Oncotarget 2015;6: 36535–50.

[16] Gorgens SW, Eckardt K, Elsen M, *et al.* Chitinase-3-like protein 1 protects skeletal muscle from TNFalpha-induced inflammation and insulin resistance. Biochem J 2014;459:479–88.

[17] Kognole AA, Payne CM. Inhibition of mammalian glycoprotein YKL-40: identification of the physiological ligand. J Biol Chem 2017;292: 2624–36.

[18] Shao R, Taylor SL, Oh DS, *et al.* Vascular heterogeneity and targeting: the role of YKL-40 in glioblastoma vascularization. Oncotarget 2015;6: 40507–18.

[19] Libreros S, Iragavarapu-Charyulu V. YKL-40/CHI3L1 drives inflammation on the road of tumor progression. J Leukoc Biol 2015;98:931–6.

[20] Giuffrida P, Pinzani M, Corazza GR, *et al.* Biomarkers of intestinal fibrosis—one step towards clinical trials for stricturing inflammatory bowel disease. United European Gastroenterol J 2016;4:523–30.

[21] Cohen N, Shani O, Raz Y, *et al.* Fibroblasts drive an immunosuppressive and growth-promoting microenvironment in breast cancer via secretion of Chitinase 3-like 1. Oncogene 2017;36:4457–68.

[22] Tschirdewahn S, Reis H, Niedworok C, *et al.* Prognostic effect of serum and tissue YKL-40 levels in bladder cancer. Urol Oncol 2014;32:663–9.

[23] Johansen JS, Jensen BV, Roslind A, *et al.* Serum YKL-40, a new prognostic biomarker in cancer patients? Cancer Epidemiol Biomarkers Prev 2006;15:194–202.

[24] Spoorenberg SM, Vestjens SM, Rijkers GT, *et al.* YKL-40, CCL18 and SP-D predict mortality in patients hospitalized with community-acquired pneumonia. Respirology (Carlton, Vic) 2017;22:542–50.

[25] Vind I, Johansen JS, Price PA, *et al.* Serum YKL-40, a potential new marker of disease activity in patients with inflammatory bowel disease. Scand J Gastroenterol 2003;38:599–605.

[26] Schmidt M, Pedersen L, Sorensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol 2014;29:541–9.

[27] Guillou L, Coindre JM, Bonichon F, *et al.* Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. J Clin Oncol 1997;15:350–62.

[28] Bojesen SE, Johansen JS, Nordestgaard BG. Plasma YKL-40 levels in healthy subjects from the general population. Clin Chim Acta 2011;412:709–12.

[29] Engellau J, Anderson H, Rydholm A, *et al.* Time dependence of prognostic factors for patients with soft tissue sarcoma: a Scandinavian Sarcoma Group Study of 338 malignant fibrous histiocytomas. Cancer 2004;100:2233–9.

[30] Altman DG, McShane LM, Sauerbrei W, *et al.* Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): explanation and elaboration. PLoS Med 2012;9:e1001216.

[31] Chen CC, Pekow J, Llado V, *et al.* Chitinase 3-like-1 expression in colonic epithelial cells as a potentially novel marker for colitis-associated neoplasia. Am J Pathol 2011;179:1494–503.

[32] Kaytan E, Yaman F, Cosar R, *et al.* Prognostic factors in localized soft-tissue sarcomas. Am J Clin Oncol 2003;26:411–5.

[33] Maretty-Nielsen K, Aggerholm-Pedersen N, Safwat A, *et al.* Prognostic factors for local recurrence and mortality in adult soft tissue sarcoma of the extremities and trunk wall: a cohort study of 922 consecutive patients. Acta Orthop 2014;85:323–32.

[34] Zagars GK, Ballo MT, Pisters PW, *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;97:2530–43.

[35] Rydholm A, Berg NO, Gullberg B, *et al.* Prognosis for soft-tissue sarcoma in the locomotor system. A retrospective population-based follow-up study of 237 patients. Acta Pathol Microbiol Immunol Scand A 1984;92:375–86.

[36] Grunhagen DJ, de Wilt JH, Graveland WJ, *et al.* Outcome and prognostic factor analysis of 217 consecutive isolated limb perfusions with tumor necrosis factor-alpha and melphalan for limb-threatening soft tissue sarcoma. Cancer 2006;106:1776–84.

[37] Aggerholm-Pedersen N, Maretty-Kongstad K, Keller J, *et al.* The prognostic value of serum biomarkers in localized bone sarcoma. Transl Oncol 2016;9:322–8.

[38] Nagano A, Ishimaru D, Nishimoto Y, *et al.* Primary bone sarcomas in patients over 40 years of age: a retrospective study using data from the Bone Tumor Registry of Japan. J Orthop Sci 2017;22:749–54.

[39] Maretty-Nielsen K. Prognostic factors in soft tissue sarcoma. Danish Med J 2014;61:B4957.

[40] Pisters PW, Leung DH, Woodruff J, *et al.* Analysis of prognostic factors in 1,041 patients with localized soft tissue sarcomas of the extremities. J Clin Oncol 1996;14:1679–89.

[41] 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;15:361–87.

[42] Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. Am J Epidemiol 2007;165:710–8.