Translational Oncology
www.transonc.com

Volume 10 Number 6 December 2017 pp. 942–948 942

A Validated Prognostic Biomarker Score for Adult Patients with Nonmetastatic Soft Tissue Sarcomas of the Trunk and Extremities

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Abstract

BACKGROUND: The prognostic value of serum biomarkers in soft tissue sarcoma (STS) is limited, and its clinical applicability is compromised by a common inability to adjust for important confounders. The aim of this study was to determine the prognostic value of pretreatment biomarkers on disease-specific survival (DSS) adjusted for confounders. *METHODS:* The study included 818 patients with localized STS. Pretreatment levels of albumin, C-reactive protein, hemoglobin, neutrophils, and lymphocytes were tested individually and combined in prognostic scores: neutrophil/lymphocyte ratio (NLR), Glasgow Prognostic Score (GPS), and Aarhus Composite Biomarker Score (ACBS) which includes all five biomarkers. Patients were randomly split into a test cohort and a validation cohort. The prognostic value of biomarkers on DSS was estimated using crude and adjusted Cox proportional hazard models. The different biomarker scores were compared using Akaike's information criteria. *RESULTS:* In the test cohort of 403 patients, all biomarkers except lymphocyte count were significant prognostic factors for DSS also after adjusting for confounders. NLR, GPS, and ACBS were independently associated with decreased survival; however, ACBS was significantly superior to NLR ($P = .02$) and GPS ($P = .002$). These findings were validated in the randomly assigned validation cohort of 415 patients. In the pooled data of 818 patients, the ACBS performed better than GPS and NLR. ACBS 2 was independently associated with decreased DSS compared to ACBS 0, hazard ratio 2.3[95% confidence interval: 1.5-3.5], $P < .001$. *CONCLUSION:* Patients with abnormal values in more than one serum biomarkers had a significant additional risk of dying compared to patients with only one abnormal value. ACBS was validated as an independent prognostic factor that is superior to both NLR and GPS.

Translational Oncology (2017) 10, 942–948

Introduction

Sarcoma is a grave diagnosis. Despite great improvements in diagnosis and treatment, the long-term survival of sarcoma patients remains unsatisfactory. The standard of care for localized soft tissue sarcoma (STS) in adults is wide surgical resection often combined with radiotherapy [1]. However, some 50% of all patients with adequate local control develop distant metastases and ultimately die from their disease [2]. The use of adjuvant chemotherapy based on traditional high-risk factors such as tumor grade and size has yielded conflicting results, so the benefit of adjuvant chemotherapy remains uncertain [3]. What is now needed is to determine other preoperative prognostic factors that will permit more accurate patient stratification and improve clinical decision-making via tailored therapy.

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Support: The study was supported by "Frits, Georg & Marie Cecilie Glud's grant", "Max & Inge Wørzner's grant", "Erland Richard Frederiksen and Wife's grant", and grants from the Danish Council for Independent Research | Medical Sciences and Aarhus University.
Conflicts of interest: None to declare.
[1] co 1. Author.
Received 20 August 2017; Accepted 14 September 2017

1936-5233/17
https://doi.org/10.1016/j.tranon.2017.09.002

It has become increasingly accepted that certain systemic inflammatory responses may play an important role in the development and progression of various cancer types. Several inflammation-based biomarkers such as hemoglobin, C-reactive protein (CRP), and some prognostic scores, such as the Glasgow Prognostic Score (GPS), neutrophil/lymphocyte ratio (NLR), and platelet/lymphocyte ratio, have shown prognostic value for many types of cancer [4–13] including sarcomas [14–22]. However, the existing studies suffer from many pitfalls. For example, the majority of studies only investigate one or two biomarkers at a time, making it impossible to study the possible interactions and relationships between the various biomarkers. Additionally, none of the existing studies adjusted for comorbidities. The presence of comorbidity is a known prognostic factor for survival and might also be closely related to abnormal biomarkers levels [23].

We have recently shown that a battery of serum biomarkers comprised of 5 proinflammatory biomarkers integrated in a prognostic score named Aarhus Composite Biomarker Score (ACBS) is prognostic for localized nonmetastatic bone sarcomas even after adjusting for various confounders including comorbidity [24].

One can assume that including 5 proinflammatory biomarkers in one scoring system such as ACBS can better capture various tumor-induced inflammatory reactions impacting the prognosis than individual biomarkers and that its value would thus not be limited to bone sarcoma but could be extended to STS and possibly other cancer types.

The aims of this study were to test the prognostic value of the selected biomarkers and scores on confounder-adjusted survival of patients with localized STS and to validate that ACBS is superior to individual markers and other prognostic scores in effectively stratifying the outcome of treatment.

Patients and Methods

The study included all 818 consecutive adult patients with a nonmetastatic STS in the extremities or trunk wall that were treated at Aarhus Sarcoma Centre, Denmark [25], between January 1994 and December 2013. Patients were excluded if they had no blood samples prior to treatment or if the blood samples were irretrievable.

The biomarkers were selected based on a literature review and the possibility of our data: hemoglobin, serum albumin, neutrophils count, lymphocytes count, and CRP. The data were obtained primarily through linkage with the clinical laboratory information system (LABKA) research database [26] or through revision of the medical files. Serum biomarker test results from 30 days preceding the date of the sarcoma diagnosis to the day before treatment were included. The biomarkers were analyzed as dichotomized categorical variables, and cutoff values were chosen based on the local reference values or previous STS studies [14,24,27–29].

Data on patients' characteristics, comorbidity, and follow-up were obtained from the Aarhus Sarcoma Registry, the National Patient Registry, the Central Population Registry, and the Cause of Death Registry, as described in a previous study [25,30–32]. Comorbidity was assessed using the Charlson Comorbidity Index [33].

Baseline characteristics were analyzed using the χ^2 and the Kruskal-Wallis test. Outcome was reported as disease-specific survival (DSS), which included deaths due to STS and deaths of patients with metastatic STS. Patients alive at the end of the study period (January 26, 2016) were censored.

Before analyzing the data, patients were randomly split into at test cohort and a validation cohort. The random split was based on year of diagnosis, sex, and age.

The biomarkers were analyzed individually as well as combined. The combined scores were NLR, GPS, and the ACBS, composed of all 5 biomarkers. Crude and confounder adjusted analyses were performed using the Cox proportional hazard model and presented as Kaplan-Meier curves. The confounder-adjusted analyses included age, tumor size, grade, histological type, depth of the tumor, as well as comorbidity. The 5-year DSS was reported using Kaplan-Meier survival estimates and compared by log-rank test. The different biomarker scores were compared using the likelihood ratio test and the Akaike's information criteria. The ACBS was then tested in a validation cohort, generated as previously described.

The bootstrapping method with 1000 iterations was used as a secondary validation of the ACBS on the pooled data to verify the robustness of the ACBS.

All tests were two-sided, and a P value ≤ .05 was considered significant. Analyses were performed using Stata, version 14.0.

The study was conducted in accordance with the Helsinki Declaration. Approval was obtained from the National Committee on Health Research Ethics, the Danish Data Protection Agency, and the Danish Health and Medicines Authority.

Results

Patients Characteristics

The study included 818 consecutive adult patients with a nonmetastatic STS in the extremities or trunk wall. This cohort was randomly divided into a test cohort (n = 405) and a validation

Table 1. Clinicopathological Characteristics in Patients with Nonmetastatic STS according to Test and Validation Cohorts (N = 818)

		Cohort		
	Total	Test Cohort	Validation Cohort	*P*
No. of patients	818	403	415	
Age (years)				
Median, (range)	60 (15-96)	60(15-93)	59(15-96)	1.00
Sex				
Female	365	179(44)	186(45)	
Male	453	224(56)	229(55)	.9
Comorbidity				
No	597	288(71)	309(74)	
Mild	75	35(9)	40(10)	
Moderate/severe	146	80(20)	66(16)	.33
Tumor size (cm)				
Median, (range)	6 (1-40)	7 (1-40)	6 (1-40)	.78
Depth				
Subcutaneous	290	147(36)	143(35)	
Subfascial	527	256(64)	271(65)	.56
Grade				
Low	192	94(23)	98(24)	
Intermediate	121	58(14)	63(15)	
High	505	251(62)	254(61)	.93
Year of diagnosis				
1994-2003	381	188(47)	193(47)	
2004-2013	437	215(53)	222(53)	.97
Treatment				
Surgery	802	391(97)	411(99)	.04
Radiotherapy	291	140(34)	151(36)	.62
Chemotherapy	38	20(5)	18(4)	.67

NOTES: P values based on the χ^2 and the Kruskal-Wallis test. Abbreviations: *Surg* = surgery, *Rt* = radiotherapy, *Ch* = chemotherapy.

cohort (n = 413). There was no difference in patients' characteristics and the distribution of important prognostic factors between the two groups (Table 1).

The Test Cohort

The median age was 60 years (range 15-93), and the male to female ratio was 1.25. The primary tumors were located at the lower extremities, trunk, and upper extremities in 52%, 34%, and 14% of the patients, respectively. The most frequent histological types were liposarcoma (20%), undifferentiated pleomorphic sarcoma (17.5%), leiomyosarcoma (17.5%), dermatofibrosarcoma (7%), synovial sarcoma (6%), and malignant peripheral nerve sheath tumor (6%). The remaining 26% comprised a combination of various less common sarcomas. The median follow-up time was 5.7 years (range: 0.1-22.0). During follow-up, 102 patients died of sarcoma, corresponding to a 5-year DSS of 78% (95% confidence interval [CI]: 76-82).+

Figure 1 shows the crude 5-year DSS according to the investigated biomarker. There was a significant difference in 5-year DSS in all individual biomarkers except lymphocytes, with the greatest difference for albumin (normal albumin: 79% [95% CI: 74-83] vs low albumin: 40% [95% CI: 22-58]) and the least for neutrophils (normal neutrophils count: 78% [95% CI: 73-83] vs high neutrophils count: 62% [95% CI: 47-74].

Patients with normal NLR had a significantly higher 5-year DSS (80% [95% CI: 77-85]) compared to patients with elevated NLR

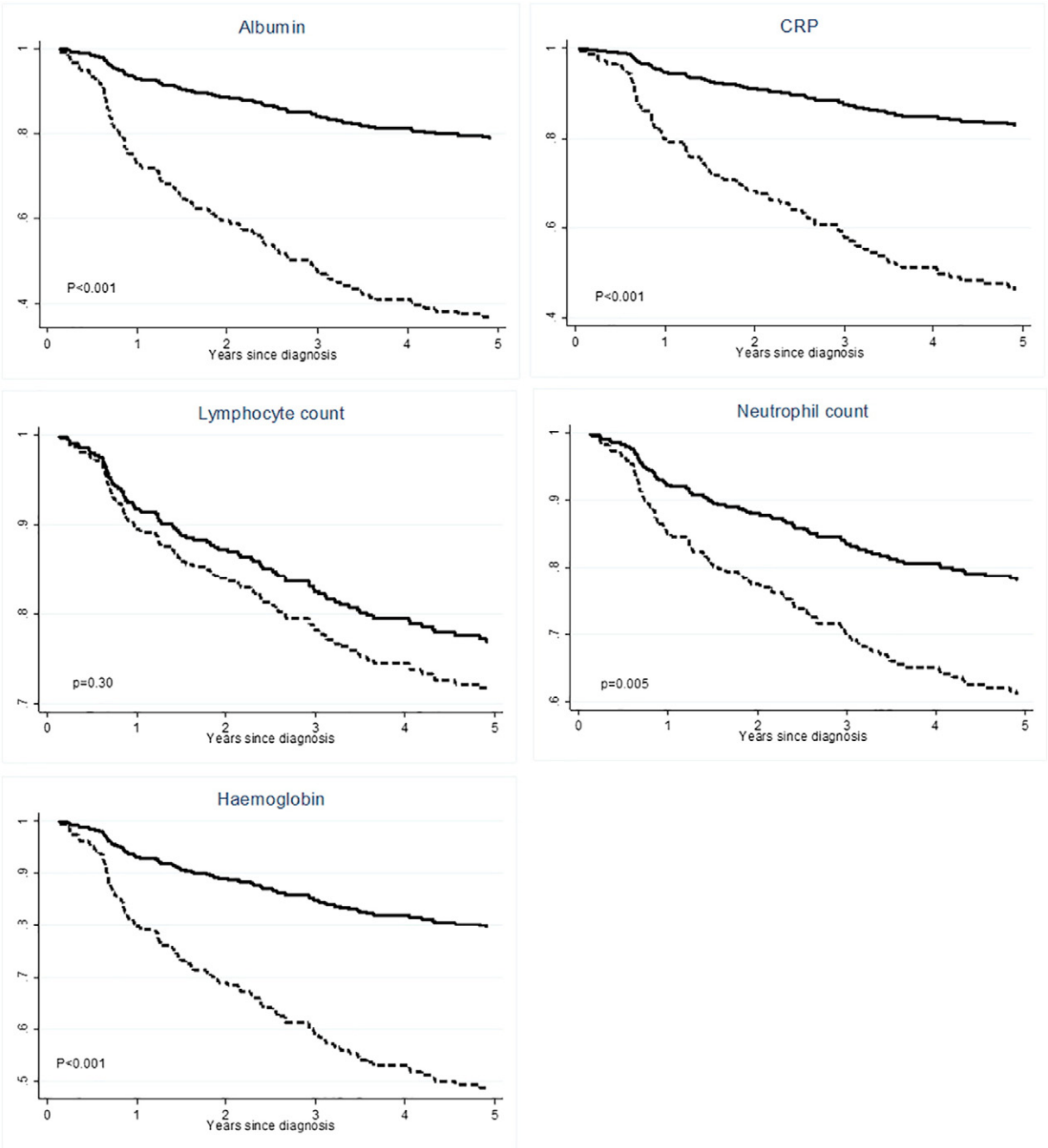

Figure 1. The DSS for individual biomarker in the test cohort, n = 403.

(60% [95% CI: 47-73]). Similarly, the 5-year DSS was 83% (95% CI: 80-86) in patients with GPS-0 compared to 64% (95% CI: 56-72) and 50% (95% CI: 35-66) in patients with GPS-1 and GPS-2, respectively.

The ACBS was then investigated, and 53% had normal values in all biomarkers (score = 0), 28% had only one abnormal value (score 1), while 19% had abnormal values in two or more biomarkers (score = 2). The 5-year DSS was 86% (95% CI: 79-90) in patients with score 0 compared to 72% (95% CI: 61-80) and 49% (95% CI: 35-62) in patients with score 1 and score 2, respectively. There was a statistically significant difference in the crude DSS between patients with score 0 and 1 (hazard ratio [HR] = 2.0 [95% CI: 1.2-3.4], P = .008) as well as between score 1 and 2 (crude HR = 2.8 [95% CI: 1.6-4.6], P < .001).

Cox proportional hazard analyses adjusted for age, comorbidity, tumor size, grade, histological type, and tumor depth confirmed that low albumin; high CRP; low hemoglobin; and elevated NLR, GPS 2, and ACBS 2 were independently associated with decreased DSS (Table 2) in the test cohort. However, the ACBS model was statistically significantly better than both the NLR and GPS models (P = .002).

Validation of the ACBS

The ACBS was validated in the randomly selected validation cohort consisting of 415 patients ACBS could stratify the outcome of the validation cohort into 3 statistically different prognostic groups with. Estimated 5-year DSS that was almost identical to the test cohort. Figure 2 shows DSS for the ACBS in the test and validation cohorts, respectively.

The crude HR in the validation cohort for ACBS score 1 was 2.0 (95% CI: 1.2-3.3), P = .009, and the crude HR for score 2 was 4.3 (95% CI: 2.5-7.2), P < .001, compared to score 0.

Table 2. Crude and Adjusted Analyses of the Importance of Biomarkers for DSS in STS Patients (n = 403)

	No. of Patients	No. of Events	Crude			Adjusted [a]		
			HR	95% CI	P [b]	HR	95% CI	P [b]
Albumin								
Normal	356	82	1			1		
Low	37	18	4.3	2.6-7.2	<.0001	2.1	1.1-3.8	.02
CRP								
Normal	281	52	1			1		
High	81	37	4.1	2.7-6.3	<.0001	1.8	1.1-3.0	.02
Hemoglobin								
Normal	346	77	1			1		
Low	54	24	3.2	2.0-5.1	<.0001	2.4	1.4-4.2	.001
Lymphocyte								
Normal	315	78	1			1		
Low	77	22	1.3	0.8-2.1	.3	1.1	0.7-1.8	.76
Neutrophil								
Normal	332	79	1			1		
High	60	21	2.0	1.2-3.2	.005	1.6	0.9-2.7	.09
NLR								
Normal	341	79	1			1		
High	51	21	3.0	1.9-4.9	<.0001	1.9	1.1-3.2	.01
GPS								
Normal	286	54	1			1		
Abnormal, score 1	53	24	3.7	2.3-5.9	<.0001	1.7	1.0-3.0	.052
Abnormal, score 2	19	10	9.2	4.6-18.2	<.0001	2.8	1.3-6.1	.009
ACBS								
Normal	189	30	1			1		
Abnormal, score 1	98	27	2.0	1.2-3.4	.008	1.6	1.0-2.8	.07
Abnormal, score 2	67	31	5.6	3.4-9.3	<.0001	2.7	1.5-4.9	.001

[a] Analyses adjusted for age, comorbidity, tumor size, grade, histological type, and depth.
[b] P values based on the Cox proportional hazard model.

Analysis of Pooled Data

The data were pooled, and an adjusted analysis for each of the 3 combined scores, NLR, GPS, and ACBS (Table 3), was then constructed.

Only 41 patients (5.5%) had the highest score in the GPS model compared to 144 (19.8%) in the ACBS. Patients with ABCS 2 and a GPS 0 or 1 had a 5-year DSS of 59% (95% CI: 48-69) and a 10-year DSS of 49% (95% CI: 37-61). Patients with an ABCS score of 2 and a GPS of 1 had a 5-year DSS of 58% (95% CI: 44-70), which is similar to GPS 1; however, their 10-year DSS was 47% (95% CI: 31-62).

Adjusting for confounders and comorbidity led to the loss of statistical significance between score 1 and 0 for both GPS and ACBS. However, ACBS score performed best evaluated by Akaike's information criteria.

A second validation of the ACBS was performed with the pooled data using the bootstrapping method. This test confirmed that ACBS 2 was associated with decreased DSS compared to ACBS 0, HR: 2.3 [95% CI: 1.5-3.5], P < .001.

Discussion

The prognostic role of biomarkers in STS has recently received increased attention. While the majority of studies investigated individual inflammatory markers, such as CRP, neutrophils, and lymphocytes, some have studied a combination of them expressed as a ratio or a simple score [14,15,17].

In this study, 5 different serum biomarkers were studied individually and in combination in a large population-based cohort of 818 patients

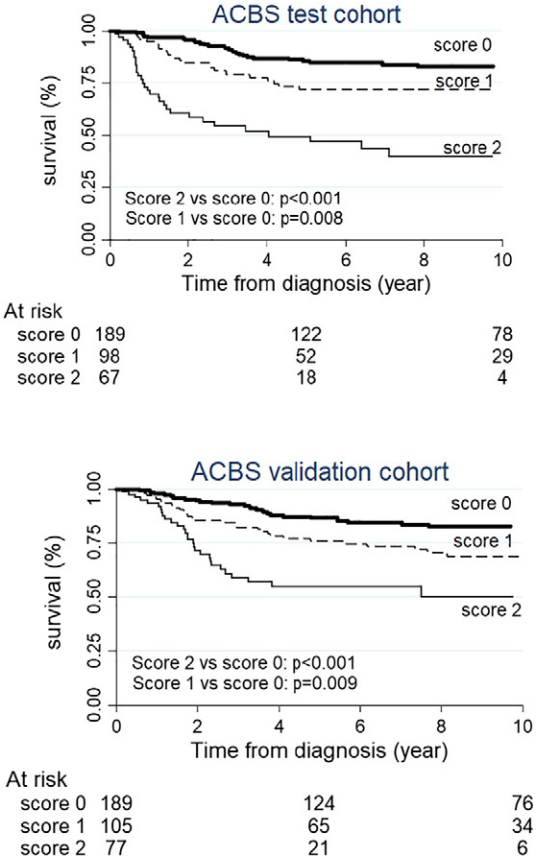

Figure 2. The DSS of the ACBS in the test and validation cohort.

Table 3. Crude and Adjusted Analyses of the Importance of Biomarkers for DSS in STS Patients (Pooled Data, $N = 818$)

	No. of Patients	No. of Events	Crude			Adjusted[a]			5-year DSS (%)	95% CI	10-year DSS (%)	95% CI
			HR	95% CI	P[b]	HR	95% CI	P[b]				
ACBS												
Score 0	378	58	1			1			86	82-89	82	78-87
Score 1	203	56	2.0	1.4-2.9	<.001	1.4	1.0-2.0	.09	74	67-79	70	63-76
Score 2	144	60	4.8	3.4-7.0	<.001	2.3	1.5-3.4	<.001	52	43-61	45	34-55
NLR												
Normal	690	151	1			1			80	77-83	76	77-83
High	103	41	2.9	2.1-4.1	<.001	1.8	1.2-2.5	.002	54	42-65	47	34-59
GPS												
Score 0	575	111	1			1			82	79-85	78	74-82
Score 1	117	45	2.8	2.0-4.0	<.001	1.4	1.0-2.1	.07	60	49-67	55	44-64
Score 2	41	20	5.9	3.7-9.6	<.001	2.3	1.4-4.0	.002	33	17-51	33	16-51

[a] Analyses adjusted for age, comorbidity, tumor size, grade, histological type, and depth.
[b] P values based on the Cox proportional hazard model.

with localized STS of extremities and trunk wall treated at the same specialized sarcoma center. To our knowledge, the combination of these biomarkers, easily measurable in clinical practice, has not previously been studied in a large, prospective cohort.

This study has many major strengths compared to other literature data. Apart from the large number of patients and being population based, all the tested parameters were prospectively measured. Furthermore, the individual linkage between the Aarhus Sarcoma Registry and the national registries provided complete follow-up and outcome reporting of all patients, as well as detailed data on comorbidity and cause of death. Data on all confounders and outcomes were collected independently of this study, and possible information bias is thus expected to be nondifferential. Most importantly, the results were validated in a randomly assigned cohort as well as by performing bootstrapping in the pooled data.

In accordance with our findings, two studies [15,16] investigating CRP reported significantly poorer DSS in patients with elevated levels, while a third study [14] reported that only a combination of elevated CRP and NLR was significant. The prognostic role of NLR has previously been investigated in STS [14,18,21]. However, to our knowledge, only two studies investigated the impact on DSS, and contrary to our findings, neither of these found a significant impact of NLR [14,21].

The existing literature on the prognostic role of lymphocyte and neutrophil counts alone is sparse, and to our knowledge, only the impact on overall survival has been investigated, finding no significant impact [19,20,22].

A small study, investigating 61 STS patients including metastatic cases, reported no significant impact of hypoalbuminemia on DSS [34]. However, the relationship was only investigated univariately, and the small number of patients included makes their result less reliable.

Our finding that anemia was an important prognostic factor corroborates the findings in a study of 376 adult STS patients with nonmetastatic disease showing anemia to be independently associated with poorer event-free and DSS [17].

The correlation between biomarker levels and cancer is complex and multifactorial. For example, it is suggested that cancer-induced circulating cytokines, such as interleukin 1 and 6, inhibit the synthesis of albumin while inducing the synthesis of acute phase proteins and hepcidin in the liver [35–37]. Hepcidin is an iron-regulating hormone, which inhibits the utilization of iron, causing anemia. Based on this suggested pathophysiological mechanism linking CRP, albumin, and anemia, one can reasonably assume that patients with abnormal levels of all three parameters may have a greater, albeit microscopic, tumor burden than patients with abnormality in only CRP. This pathophysiological pathway, however, is not the only one, and other pathways involving other biomarkers such as leukocyte count are evident.

Combining more than one biomarker into a composite biomarker score may therefore lead to a more precise prognosis prediction than using a single biomarker since it may measure various biological mechanisms adversely affecting survival.

ACBS demonstrated a strong and clinically relevant correlation with DSS, especially in patients with more than one abnormal value. The impact on survival is assumed to be through metastatic disease, whereby the abnormal biomarker score reflects the presence of early, subclinical metastatic spread. Identifying these patients at an earlier disease stage might enable more aggressive treatment to prevent early death.

In our previous study of serum biomarkers in 172 patients with nonmetastatic intermediate and high-grade bone sarcomas, only elevated CRP and anemia were independent prognostic factors for confounder-adjusted DSS, while both neutrophils and hypoalbuminemia were also independently prognostic in the current study. The difference may reflect a true biological difference between bone and soft tissue sarcomas but can also be the result of the smaller number of patients in the bone sarcomas study [24]. However, it is important to underline that, regardless of this difference, using the five-biomarker–based ACBS system was useful as prognostic tool in both bone and soft tissue sarcomas.

NLR divided patients into two groups with only 13% stratified to the poor prognostic group, while ACBS, and GPS stratified the patients into 3 groups which is clinically preferable as it theoretically allows for a more refined stepwise treatment intensification strategy. Comparing GPS and ACBS is a difficult task since they may pick different patient groups.

Only 41 patients (5.5%) had the highest score in the GPS system compared to 144 (19.8%) in the ACBS. This difference may suggest that a substantial number of patients with potentially poor prognosis may be missed if one would rely solely on GPS. Indeed, analyzing DSS of the subgroup of patients with the highest ABCS score of 2 but a normal or intermediate GPS (0 or 1) showed a 5- and 10-year DSS similar to what is expected based on their ACBS score rather than GPS. Therefore, it seems that ACBS score can capture larger number of patients with a true poor prognosis and can better predict their long-term survival.

ACBS is a data-generated prognostic tool that is designed on the assumption that an optimal risk stratification biomarker scoring

system needs to combine as many biomarkers as needed to reflect the multiplicity of pathophysiological pathways and biological mechanisms contributing to decreased survival. ACBS is presented as a model that can be expanded by adding other biomarkers such as serum sodium as dictated by the data.

In conclusion, we identified elevated CRP, anemia, and hypoalbuminemia as independent risk factors for early death in localized STS patients. Combining these biomarkers in the five-biomarker ACBS model could better stratify treatment outcome than GPS or NLR. The prognostic value of ACBS detected in a test cohort group was validated in a similarly sized randomly assigned validation cohort. ACBS could identify patients with nonmetastatic STS eligible for more aggressive diagnostics and treatment and may be considered in treatment decision making in conjunction with other known clinical or pathological prognostic factors.

Acknowledgements

The study was supported by "Frits, Georg & Marie Cecilie Glud's grant", "Max & Inge Wørzner's grant", "Erland Richard Frederiksen and Wife's grant", and grants from the Danish Council for Independent Research | Medical Sciences and Aarhus University.

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