SAHLGRENSKA ACADEMY

Lactate dehydrogenase and albumin as predictive markers for treatment response in sarcoma patients

Degree Project in Medicine

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Programme in Medicine

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1. Abstract

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Introduction: Sarcomas are rare cancer diseases. During treatment and follow up patients are primarily monitored with radiology. Due to lacking sensitivity, detection of early relapses is challenging using this method. It also exposes patients for radiation doses that might be harmful. Lactate dehydrogenase (LDH) and albumin are biomarkers that have been shown to indicate increased overall survival in sarcoma patients; additionally, they are measured in clinical routine. No prior study, in our knowledge, have investigated these markers as predictive factors for treatment outcome.

Aim: The aim of this study was to evaluate baseline LDH and albumin as predictive markers for treatment response at first radiology evaluation. The second aim was to explore if any other clinical or patient related factor could be associated with the baseline levels of LDH and albumin. In a subset of patients, we also explored how LDH, and albumin levels varied over time.

Methods: In a retrospective observational study we analysed 33 patients diagnosed with different types of sarcomas. Patients were monitored from start of treatment until their first radiology evaluation. Treatment response was evaluated from radiologic or histologic examinations. Correlation analyses were performed between different subgroups.

Results: There was no significant difference in LDH ($p = 0.211$) and albumin levels ($p = 0.779$) between responders and non-responders at baseline. We found a correlation between LDH and aspartate aminotransferase (AST), alkaline phosphatase (ALP), sarcoma subtype

and lung metastases, respectively. Further, correlations were observed between albumin and creatinine and performance status, respectively. The levels of LDH and albumin correlated to some degree to clinical outcome over time.

Conclusions: Baseline LDH and albumin levels display no value as predictive factors for treatment response in the studied patient cohort.

Keywords: lactate dehydrogenase, albumin, predictive, sarcomas, biomarkers.

2. Introduction

2.1. Cancer and sarcomas

2.1.1. Cancer

Cancer is a disease characterised by abnormal cell proliferation and invasion that can occur in almost all cell types in the body. Depending on the subtype of cancer, the clinical features, prognosis and treatment varies (1). Cancers are most commonly caused by mutations which result in properties necessary for tumour development, such as dysregulated growth, resistance to cell death, activation of invasion and immortal replication (2).

Approximately 65 000 Swedes are diagnosed with cancer every year (3), and statistics estimate that one in three Swedes will be diagnosed with cancer during their life time (4). Furthermore, cancer is the second most common cause of death in Sweden (5). The five most common subtypes of cancer in Sweden are prostate cancer, breast cancer, colorectal cancer, lung cancer and malignant melanoma. Together, they constitute approximately 50% of all new diagnosed cases of cancer. The remaining 50% consist of more rare subtypes of cancers (3). 90% of all malignancies arise from epithelial tissue and are referred to as carcinomas (6). The most common tumours in the non-epithelial group of malignancies are leukaemia, lymphomas, germ cell tumours and sarcomas (1).

2.1.2. Sarcomas

Sarcomas are rare malignancies, arising from mesenchymal tissue (1). Sarcomas are subdivided into soft tissue and bone sarcomas, where soft tissue sarcomas accounts for ¾ and bone sarcomas for ¼ of all sarcomas (4). Sarcomas account for less than 1% of all malignancies in the adult population (7). However, sarcomas are overrepresented in paediatric populations where it accounts for 10% of all cancers (8). Moreover, when comparing soft tissue sarcomas to their benign relatives, benign mesenchymal tumours, the harmless variants

are more than a hundred times more common (7). The overall 5-year survival rate for sarcomas are 68.5% for men and 63.3% for women (4).

Generally, the incidence of soft tissue sarcomas increases with age and they are mainly located to extremities (7). More than 50 histologic subtypes of soft tissue sarcomas exists (9). The two most common forms are liposarcoma and leiomyosarcoma (10).

The incidence of bone sarcomas is age-dependent and on population level two incidence age-peaks can be distinguished – one in the second decade of life and the other after the age of 65. The most common location for bone sarcomas is the knee, followed by the pelvis (7). In the latest WHO Classification of Tumours of Soft Tissue and Bone, 28 different malignant subtypes of bone sarcomas are included (7). The most common types of bone sarcomas are chondrosarcoma, osteosarcoma, and Ewing sarcoma (10).

In Sweden, a total number of 304 and 87 individuals were diagnosed with soft tissue and bone sarcomas respectively, in 2018. In the specific region of Västra Götaland, where this study was conducted, 79 cases of soft tissue sarcomas and 12 cases of bone sarcomas were reported 2018 (11).

2.1.3. Treatment of Sarcomas

Sarcomas are generally treated with a combination of surgery, chemotherapy, and radiation therapy. Patients are diagnosed, treated, and monitored at designated regional sarcoma centres. For soft tissue sarcomas the aim is, if possible, to treat patients with radical surgery. Thereafter, if necessary, adjuvant treatment with radiation therapy and/or chemotherapy is given. However, evidence for beneficial effect of adjuvant therapy is weak. Bone sarcomas are mostly treated with surgery and neoadjuvant or adjuvant chemotherapy. The sensitivity for chemotherapy varies between different sarcoma subtypes. Patients with metastatic disease is often treated with chemotherapy and/or targeted therapy (12).

2.1.4. Prognostic factors for sarcomas

Several prognostic factors for sarcomas have been investigated. These include tumour characteristics, patient characteristics as well as biochemical characteristics. The prognostic factors have been studied on both large and small cohorts with individual as well as multiple sarcoma subtypes and focus on outcomes such as risk of metastases, local recurrence, and survival. Among soft tissue sarcomas, a few studies have been made on a thousand patients or more. These have shown similar as well as conflicting results regarding prognostic factors for soft tissue sarcomas (13-16).

Consistently, the studies have shown that the risk for developing metastatic disease increased with increasing tumour grade, size, and depth (13, 15). Negative surgical marginals and treatment with radiotherapy were factors that decreased the risk for local recurrence (15, 17). Greater tumour size, single modality treatment (18), tumour necrosis, high grade tumours (14), higher age and male sex were linked with poorer overall survival (16).

Moreover, a variety of laboratory tests have shown to be correlated with poorer survival, for example, elevated C-reactive protein (CRP) (19, 20), elevated Alkaline phosphatase (ALP) (20) and low Haemoglobin (Hb) levels (18).

Prognostic factors for bone sarcomas have been investigated in several studies, however only a few factors have consistently been shown to be independent prognostic factors for survival. In general, for bone sarcomas, age over 40 years, higher tumour grade, metastases and non-surgical treatment are correlated with poorer survival (21).

Regarding laboratory tests elevated ALP and elevated CRP have been shown to indicate poorer prognosis in osteosarcoma (22). Elevated levels of LDH have shown conflicting results as a prognostic factor for survival in both Ewing sarcoma and osteosarcoma (23, 24).

At last, for cancer patients as a group, a higher grade on the Eastern Cooperative Oncology Group Performance Status (ECOG PS, Appendix table 1) has been shown to indicate a poorer prognosis (25). In clinical practice, the scale is used as a tool for decisions about treatment intent of oncology patients and to give an estimation of patients' overall wellbeing.

2.1.5. Monitoring sarcoma patients

During oncologic treatment and follow up, sarcoma patients are monitored with clinical examinations, radiology, and laboratory tests, according to national standardised protocols and local routines (12). Follow up interval depends on subtype and tumour grade. However, most patients are followed up every third month the first three years and thereafter every sixth to twelfth month, up to ten years (12). In clinical practice the patients often undergo radiology, consisting of either or both Magnet Resonance Imaging (MRI) and Computer Tomography (CT).

Follow up with radiology is convenient and can provide information about relapses among other things. However, there are limitations with radiology. First, both MRI and CT are ineffective for lesions measuring under 3 millimetres (26, 27). This could cause a delay of the detection of relapses and potentially affect the patient's prognosis. Secondly, radiation can induce secondary cancer. Sarcoma patients could be affected by radiation from both evaluating radiology examinations (CT scans, X-ray investigations) and in some cases from radiotherapy. While the risk of radiology-induced cancer is minimal for the individual person and is justified by the diagnostic need, it is a larger problem when considering the issue on population level. Since sarcoma patients undergo radiology multiple times and often at young age and since many sarcoma patients receive radiation therapy as part of their treatment, this increases the risk for secondary cancers (28). Third, it has been shown that early tumour volume response does not necessarily correlate with survival rates (29). Therefore, there is an urge for biomarkers that could indicate treatment response at an earlier stage.

2.2. Biomarkers, lactate dehydrogenase and albumin

2.2.1 Biomarkers

According to the Biomarkers Definitions Working group a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (30). The origin can be molecular, histologic, radiographic or physiologic (31). Molecular biomarkers can for example be measured in tissue, blood, plasma, or urine (32). They often consist of proteins, metabolites, or DNA (32, 33). Biomarkers can be used for different purposes, like diagnostics, prognostic information, treatment prediction, response evaluation or disease monitoring during treatment and follow up. The same biomarker can be used for different purposes, depending on the context (31).

Research in cancer biomarkers is a vast and diverse research field, yet only a few biomarkers have shown clinical utility. One example is Prostate Specific Antigen (PSA), which is a biomarker in clinical use for screening and monitoring of prostate cancer. Another is the use of alpha feto-protein, LDH and beta-HCG in monitoring of germ cell tumours (32). No tumour specific biomarkers are currently available for diagnostics, prognostic evaluation, or treatment monitoring of sarcomas. However, the unspecific tumour marker LDH is often monitored in clinical routine.

2.2.2. Lactate dehydrogenase

Lactate dehydrogenase (LDH) is a catalysing enzyme in energy metabolism in all cells. The enzyme can be found in all organisms in nature. It catalyses the reversible reaction of pyruvate into lactate with NAD+ as a cofactor, a reaction that depending on its direction generate energy in both aerobic and anaerobic environments (34). The enzyme has different isoforms, which are built of different combinations of the two major subunits A and B.

Generally, subunit A functions best in anaerobic environments, while subunit B prefer aerobic surroundings (35). LDH can be found in the cytosol, the mitochondria as well as in the cell nuclei. This varies between isoforms and the tissue location of the isoform (34).

LDH is released into the bloodstream when a tissue or organ is damaged, and its cells are being destroyed. It can therefore indicate a vast array of different illnesses. Serum LDH can be elevated in patients with cancer as a result of tissue destruction from growth of the tumour (34). Especially the isoform LDH-A has been found to be highly expressed in tumour tissue (36). Elevated serum levels of LDH have been shown to indicate poor prognosis in various forms of cancer (35).

Regarding the value of LDH as a prognostic biomarker for sarcomas, the research results vary. It has been shown that a high level of LDH indicate poor disease-free survival and overall survival in angiosarcoma and Ewing sarcoma (37, 38). However, in another study this was not a significant factor in multivariate analysis (39). Equally, LDH as prognostic factor for osteosarcoma has shown conflicting results (24). LDH levels has been shown to vary with disease stage. It is more common with elevated LDH in patients with metastatic disease (39). Moreover, elevated LDH levels together with hypoalbuminemia have been shown to be more common among patients with metastatic disease (37).

2.2.3. Albumin

Albumin is a protein found in plasma. It has several functions, the most vital is sustaining the colloid osmotic pressure in the vascular system (40). It also plays a role in inflammation. It acts as a negative acute phase protein, indicating that its levels are reduced upon inflammation. The changes of acute-phase proteins in the body can be induced by different inflammatory conditions, such as infections, traumas and cancer (41). A low pre-treatment albumin level is a negative prognostic factor for different cancer types (42).

Albumin is produced by hepatocytes. Main regulators of production are the vascular system's colloid osmotic pressure, nutritional status, and hormones. Albumin is mainly degraded in organs, yet a small amount is lost in the digestive tract and urinary system (40).

Albumin is regarded as a prognostic factor for survival in patients with localised and metastatic sarcoma. Low pre-treatment levels indicate poorer prognosis (19, 37, 43, 44). However, no differences in albumin levels have been found between metastatic and localised disease (43). At the same time hypoalbuminemia in sarcoma patients has also been associated with higher incidence of systemic symptoms, and incidence of metastases. These factors all indicate poorer prognosis (44).

No thorough explanation for the cause of hypoalbuminemia in cancer has been found, however, two alternative explanations exist. One theory is that production of systemic cytokines such as tumour necrosis factor (TNF), secreted as a response to inflammation induced by the tumour, downregulates the liver's production of albumin, thus leads to hypoalbuminemia. The other is that TNF increases the capillaries permeability, hence leading to albumin leakage from plasma to the interstitium. Interestingly, it is hypothesised that in initial stages of tumour development, albumin production is increased because of the tumour's stimulation of albumin synthesis. At later stages of cancer and with concomitant inflammation and malnutrition, albumin levels generally decrease (40).

2.2.4. Measurement of LDH and albumin at Sahlgrenska University Hospital

At Sahlgrenska University Hospital the combined amount of all LDH-isoenzymes in serum are measured with enzymatic photometric tests. The normal range of LDH for people aged between 18 and 70 is 1.8 to 3.4 μkat/L. At age over 70, the normal interval is determined to be 1.9-4.2 μkat/L (45).

Albumin is measured with photometry and immunoturbidimetry. The reference values for adult patients are separated into three groups depending on age. For adults between 18 to 40 normal values are between 36-48, for patients between 41 and 69 the normal range is between 36 to 45 and for patients at the age of 70 and older the normal range is between 34-45 grams/Litre (g/L) (46).

2.3. The importance of predictive biomarkers for treatment response

The heterogeneity and the low number of patients in the population of sarcoma patients, together with multiple histologic subtypes, is a clinical challenge. Little is known about subtype specific therapy and predictive factors in different subtypes of sarcomas. Therefore, the monitoring of sarcoma patients would benefit from the use of biomarkers for evaluating treatment response. The use of biomarkers as an alternative or guidance for when to use radiology, could prevent from excess use of radiology and make radiology more accessible for the overall population. No tumour specific biomarkers are currently available for diagnostics, predictive evaluation, or treatment monitoring of sarcomas. However, the unspecific tumour marker LDH is often monitored in clinical routine. Moreover, albumin is also monitored in clinical routine to evaluate the patients nutritional and overall status. No prior studies have been conducted investigating the predictive value of baseline LDH or albumin to treatment response in sarcomas.

3. Aim

The aim of this study was to investigate the predictive value of baseline LDH and albumin levels to treatment response at first follow up evaluation, in patients with sarcomas. Additionally, we aimed to see if there was any clinical or patient related factor that could explain differences in baseline levels of albumin and LDH. We also wanted to explore in a subset of patients how LDH and albumin levels varied over time.

3.1 Research questions

1. Does baseline LDH and albumin differ between patients who respond versus not respond at first follow up after initiation of treatment?

2. Are there any factors, for example sarcoma subtype, metastatic disease, poor ECOG PS, affected kidney- or liver function, that correlate to baseline levels of LDH and/or albumin?

3. Do LDH and albumin reflect the tumour activity and treatment response over time in patients with sarcomas?

4. Material and Methods

4.1. Study design

This study was designed as a descriptive retrospective observational study. In addition, an explorative analysis of factors affecting LDH and albumin was performed.

4.2. Study population

The patients included in this study were retrieved from the Sarcoma Test Study, that was initiated in 2016. It is a longitudinal prospective study which includes patients diagnosed with sarcomas in the Region of Västra Götaland. The study follows patients over time and collects blood samples during their treatments. The aim of the study is to identify more accurate biomarkers to monitor the disease. On average, one or two patients are included every week. In February 2021, when this study was initiated, a total of 70 patients were included in the Sarcoma Test Study.

4.3. Inclusion criteria

Patients included in the sarcoma test study, receiving oncological treatment (either chemotherapy, targeted therapy, or radiation therapy) at Sahlgrenska University Hospital, with baseline levels of either LDH or albumin and with eligible radiologic or histologic response evaluations were included. If baseline radiology exceeded six weeks prior to start of treatment, patients were excluded. Six weeks were chosen since this is a commonly acceptable limit in clinical praxis. Patients who were only treated with surgical resection were excluded, except for patients that had not been radically excised and were receiving adjuvant therapy. Radiologic response evaluation was performed at first radiology evaluation. For patients receiving neoadjuvant chemotherapy prior to surgery, histologic evaluation of the resected tumour, according to pathologist report, was used as evaluation. In those cases, radiologic examination was not considered, except if patients developed distant metastases.

4.4. Data collection

Data were collected from the patients' medical records in the electronic journal system Melior and associated systems, for example laboratory and radiology programs. Data were gathered about patient characteristics (age, diagnosis, comorbidity, earlier medical events, earlier cancer diagnosis, and whether the patient was alive or not). Characteristics of patients' diseases were also collected, which included date and type of treatments, disease stage, local for metastases, radiologic responses, laboratory tests, the patients' own evaluation of their performance status (ECOG) before the start of treatment. Apart from LDH and albumin, data about Creatinine, Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were collected. Dates for all events were recorded. All available data from the time of diagnosis to present were collected. The data was compiled in an Excel sheet. Patients were anonymised and given a study ID.

4.5. Variables

Response to treatment was calculated from radiologic response according to the Recist-criteria guidelines (47) or histologic response, according to pathologist-reported statement. Patients were radiologic responders if they had radiologic complete response (CR) or partial regression (PR). Stable disease (SD) and progressive disease (PD) were classified as non-responders. The radiologic response was evaluated from the radiologist's statement. If this was not clearly written, measurement in relevant radiologic examinations was performed according to the Recist criteria guidelines (47). If patients had been classified as a responder in either radiologic or histologic examination, they were classified as responders.

Self-estimated ECOG was collected from a health declaration that patients fill out at their first visit to the oncology department. The ECOG classification is shown in appendix. In two cases the patients had filled out two different ECOG measurements at the same time, the lowest

value was then chosen for the analysis, since this was believed to be the most clinically relevant interpretation. For the analysis, patients were separated in soft tissue sarcomas or bone sarcomas depending on their sarcoma subgroup. Finally, the levels of LDH, albumin, AST and ALP were classified as low, high, or normal depending on the cut off levels used at the laboratory at Sahlgrenska University Hospital (45, 46, 48, 49).

4.6. Statistical analysis

To illustrate differences and investigate whether trends could be seen, box plots with different variables were performed. Student's t-test and Mann-Whitney U-test were performed to identify potential significant differences between groups. Correlations between continuous variables were analysed with Spearman's test for non-linear data. The statistics were performed in IBM SPSS Statistics 26. The timelines for individual changes in lactate dehydrogenase and albumin were constructed in GraphPadPrism.

5. Ethics

This report was conducted on patients included in the sarcoma test study. The sarcoma test study has been granted ethical approval from the Regional Council of Ethics Approval (Dnr: 485-16, and Dnr: T-795-16). The student received approval to access the medical records from the director of the Oncology Department at Sahlgrenska University Hospital.

6. Results

6.1. Study population

A total of 70 patients were eligible for this study. Eleven were excluded as they only underwent surgery, and another 16 were excluded since their tumours had been radically excised prior to adjuvant oncological therapy. Two patients were excluded since they had received their treatment at other hospitals than Sahlgrenska University Hospital. Apart from this, two patients were excluded since there was no follow up radiology. Six patients were excluded since their baseline radiology exceeded six weeks before start of treatment. Ultimately, 33 patients were eligible for analysis (**Figure 1**).

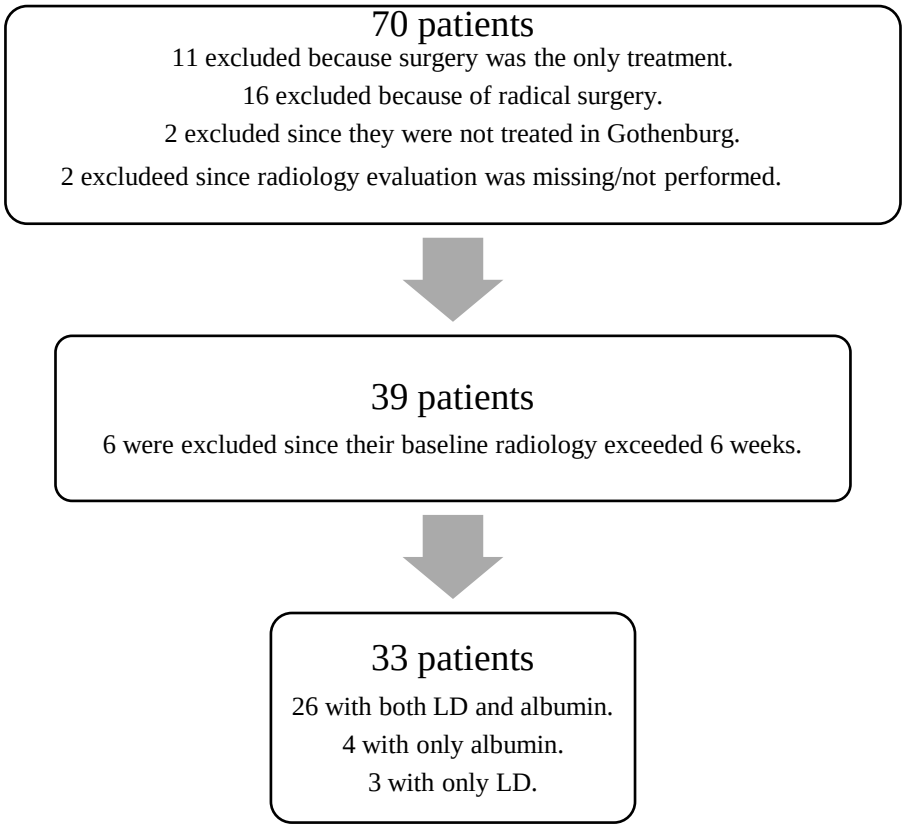

Figure 1. Flow diagram of study selection procedure.

Patient baseline characteristics were summarised in **Table 1** and **2**. LDH and albumin levels at baseline were shown in **Figure 2**. Most of the study population were under 70 (85%), were treated for a primary tumour (85%) and were men (61%). 19 patients (58%) had metastatic

disease, while 14 patients (42%) had localised disease. Almost all patients were treated with chemotherapy (88%). The median age of the study population was 42, it varied between 19 and 84. The median follow up time was 72 days, with a variation between 31 and 121 days. Data regarding sarcoma subtypes and distributions of LDH and albumin are shown in appendix (Appendix, Figure 1-3).

Table 1, baseline characteristics		Total[a]	Responder[b]	Non-responder[b]
Sex	Female	13 (39.4%)	2 (15.4%)	11 (84.6%)
(n, %)	Male	20 (60.6%)	7 (35%)	13 (65%)
Age	<70	28 (84.8%)	8 (28.6%)	20 (71.4%)
(n, %)	>70	5 (15.2%)	1 (20%)	4 (80%)
Alive	Yes	27 (81.8%)	7 (25.9%)	20 (74.1%)
(n, %)	No	6 (18.2%)	2 (33.3%)	4 (66.6%)
Type of sarcoma	Soft Tissue Sarcoma	23 (69.7%)	7 (30.4%)	16 (69.6%)
(n, %)	Bone sarcoma	10 (30.3%)	2 (20%)	8 (80%)
Disease stage	Metastatic disease	19 (57.6%)	6 (31.6%)	13 (68.4%)
(n, %)	Localised disease	14 (42.4%)	3 (21.4%)	11 (78.6%)
Lung metastases	No	23 (69.7%)	5 (21.7%)	18 (78.3%)
(n, %)	Yes	10 (30.3%)	4 (40%)	6 (60%)
Bone metastases	No	29 (87.9%)	9 (31%)	20 (69%)
(n, %)	Yes	4 (12.1%)	0	4 (100%)
Metastases in abdomen	No	28 (84.8%)	8 (28.6%)	20 (71.4%)
(n, %)	Yes	5 (15.2%)	1 (20%)	4 (80%)
Other metastases	No	26 (78.8%)	8 (30.8%)	18 (69.2%)
(n, %)	Yes	7 (21.2%)	1 (14.3%)	6 (85.7%)
Primary treatment	Chemotherapy	29 (87.9%)	7 (24.1%)	22 (75.9%)
(n, %)	Targeted therapy	2 (6%)	0	2 (100%)
	Radiation therapy	2 (6%)	2 (100%)	0
Treatment intent	Neoadjuvant	19 (59.4%)	7 (36.8%)	12 (63.2%)
(n, %)	Palliative	13 (40.6%)	2 (15.4%)	11 (84.6%)
ECOG PS	0-1	17 (77.3%)	5 (29.4%)	12 (70.6%)
(n, %)	>1	5 (22.7%)	1 (20%)	4 (80%)
	Total number	**33**	**9**	**24**

[a] percent values are calculated from the total population.
[b] percent values are calculated within each subgroup.

Table 2, mean and median at baseline according to treatment response	Treatment response			
	Responder		Non-responder	
	Mean	Median	Mean	Median
LDH	2.9	2.5	4.5	2.9
Albumin	41	44	40	40

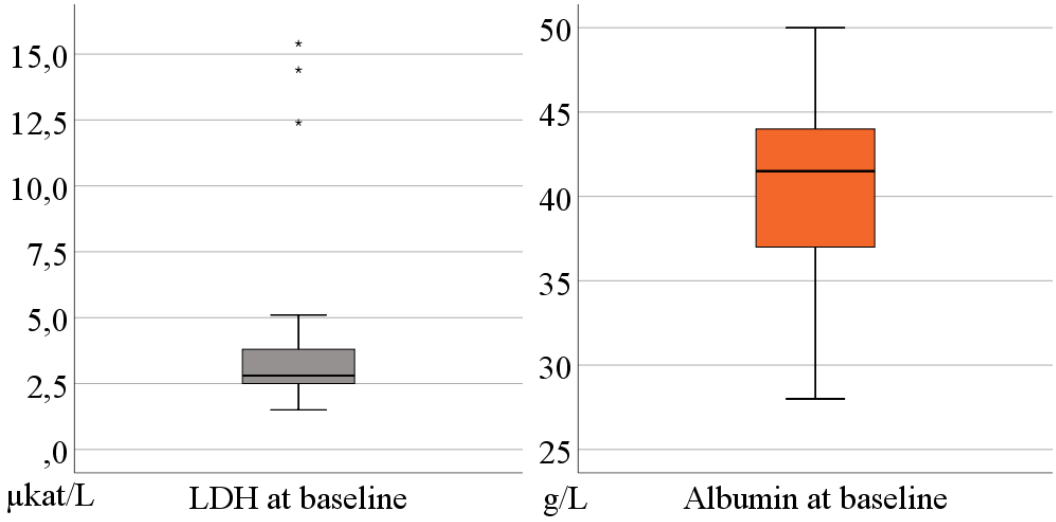

Figure 2. LDH and albumin at baseline

6.2. Responders and non-responders

Twelve patients had histologic evaluation of treatment response; 30 patients had radiologic evaluation of treatment response. Nine patients had overlapping histologic and radiologic results. The treatment response was classified consistently in all except two patients. One of these patients had radiologic response as non-responder and histologic response as responder. The other was classified as responder according to radiology evaluation, but not histologic evaluation. In total, nine patients classified as responders and 24 patients classified as non-responders. One patient over 70 years of age classified as responder, all other patients over 70 years of age were non-responders. Among both responders and non-responders, a majority had metastatic disease. Only two patients with bone sarcomas were responders. None of the responders had received targeted therapy, in contrast all patients who received radiation therapy were responders. Two patients with palliative treatment intent were responders (**Table 1**).

There was no significant difference in median baseline LDH levels between responders and non-responders (**Table 3, Figure 3**). There was no significance difference in mean value of baseline albumin between the two groups (**Table 4, Figure 4**).

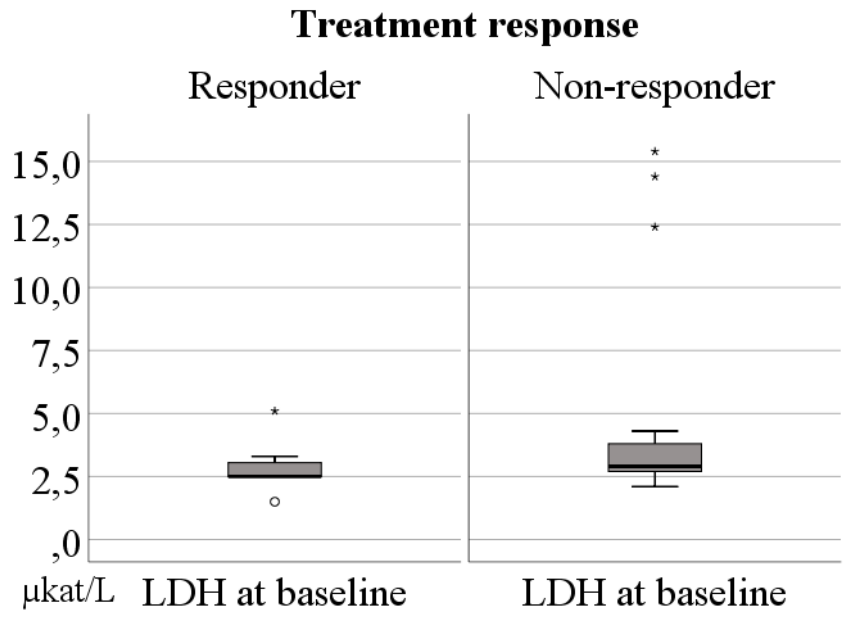

Figure 3. Baseline LDH levels for responders and non-responders respectively.

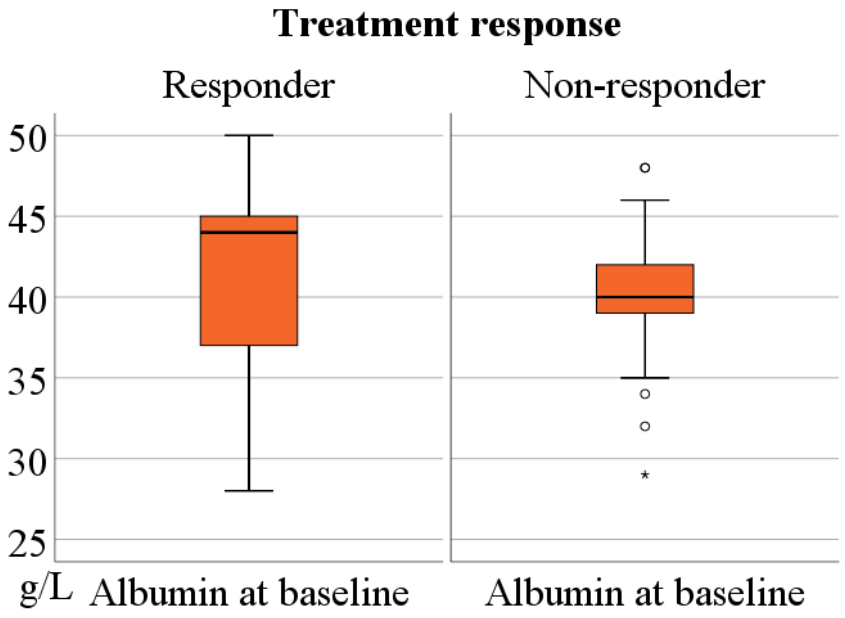

Figure 4. Baseline albumin levels in responders and non-responders.

6.3. LDH

29 patients had LDH measured before start of treatment. The median value of LDH was 2.8 µkat/L, it varied between 1.5 and 15.4. Baseline LDH levels for various subgroups were showed in **Table 3**. LDH was significantly elevated in patients with bone sarcomas and for patients with lung metastases. LDH had a positive correlation with AST and ALP. The correlation coefficient of LDH and AST were 0.430 (p = 0.02, **Table 4**). There was a

significant elevation of LDH levels between patients with high and normal AST (p = 0.013, **Figure 8, Table 5**). LDH and ALP had a correlation coefficient of 0.539 (p = 0.003, **Table 4**). LDH levels were significantly different in patients with elevated ALP compared to normal ALP (p = 0.012, **Figure 8, Table 5**). There was no correlation between LDH and age, creatinine, or ALT (**Table 4**).

Table 3, baseline LDH levels according to subgroups			Count	Median	Percentile 25	Percentile 75	Minimum	Maximum	p-value
LDH at baseline	Response to treatment	Responder	9	2.5	2.5	3.3	1.5	5.1	0.211
		Non-responder	24	2.9	2.7	3.8	2.1	15.4	
	Sex	Female	13	2.7	2.5	3.5	1.5	15.4	0.457
		Male	20	2.8	2.5	3.9	2.4	14.4	
	Age	<70	28	2.8	2.5	3.5	1.5	15.4	0.326
		>70	5	3.4	2.9	3.9	2.7	3.9	
	Type of sarcoma	Soft Tissue Sarcoma	23	2.8	2.5	3.1	1.5	12.4	**0.045**
		Bone sarcoma	10	3.8	2.8	5.1	2.1	15.4	
	Disease stage	Metastatic disease	19	3.0	2.8	3.9	2.4	15.4	**0.084**
		Localised disease	14	2.7	2.5	3.8	1.5	12.4	
	ECOG PS	0-1	17	2.9	2.5	3.3	1.5	5.1	0.711
		>1	5	2.7	2.4	3.5	2.4	3.5	
	Lung metastases	No	23	2.7	2.5	3.0	1.5	12.4	**0.004**
		Yes	10	3.9	3.3	14.4	2.8	15.4	
	Bone metastases	No	29	2.8	2.5	3.8	1.5	14.4	0.145
		Yes	4	3.4	3.0	9.5	2.8	15.4	
	Metastases in abdomen	No	28	2.8	2.6	3.9	1.5	15.4	0.325
		Yes	5	2.7	2.5	3.0	2.4	3.2	
	Other metastases	No	26	2.7	2.5	3.8	1.5	14.4	0.268
		Yes	7	3.0	2.8	3.2	2.8	15.4	

Significant values are marked in bold, underlined data are shown in boxplots below (**Figure 5-7**).

Table 4, Spearman's correlation for LDH level at baseline according to linear variables.		
Factor	Spearman's correlation coefficient (r_s)	p-value
Age	0.107	0.580
ALT	-0.098	0.614
AST	0.430	**0.02**
ALP	0.539	**0.003**
Creatinine	0.023	0.907
Albumin	-0.028	0.890

Significant values are marked in bold.

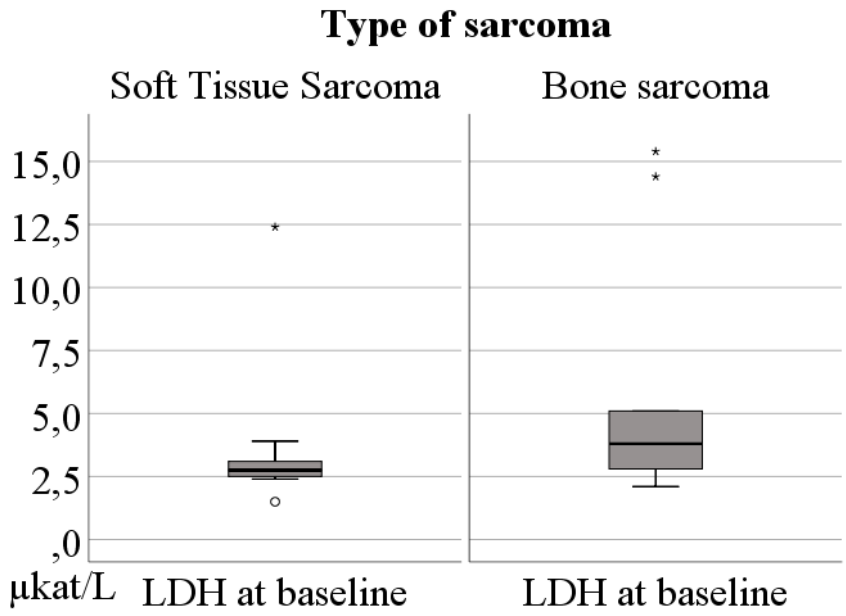

Figure 5. Difference in baseline LDH levels between patients with soft tissue sarcoma and bone sarcoma.

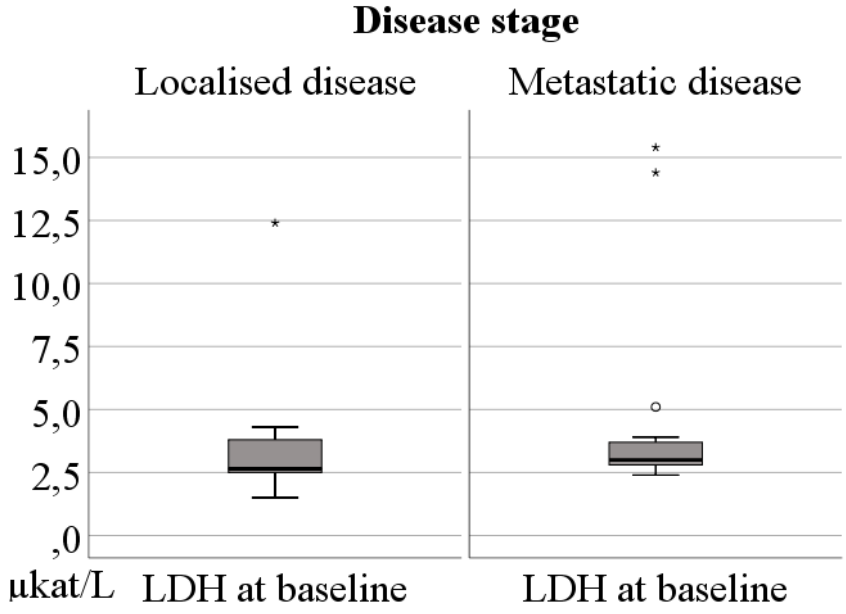

Figure 6. LDH levels in metastatic disease compared to localised disease.

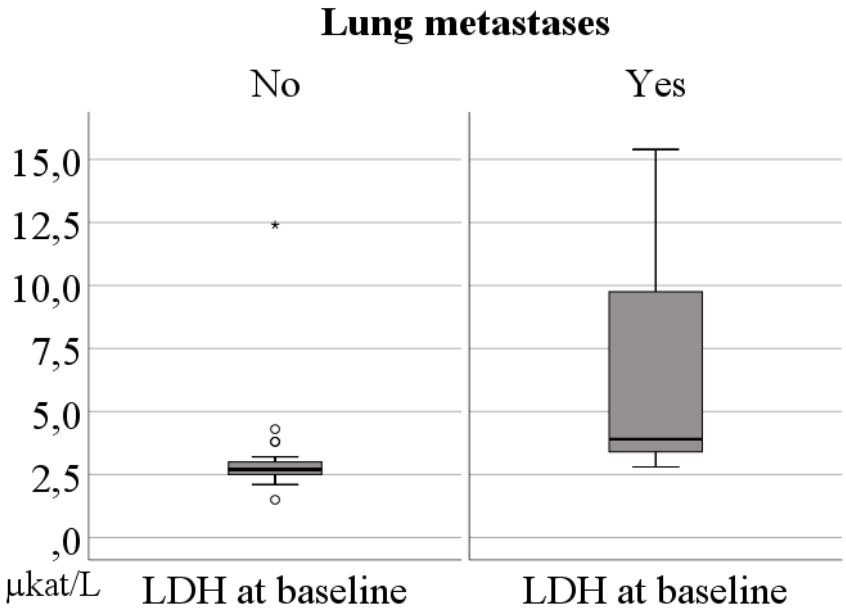

Figure 7. LDH levels in patients with lung metastases.

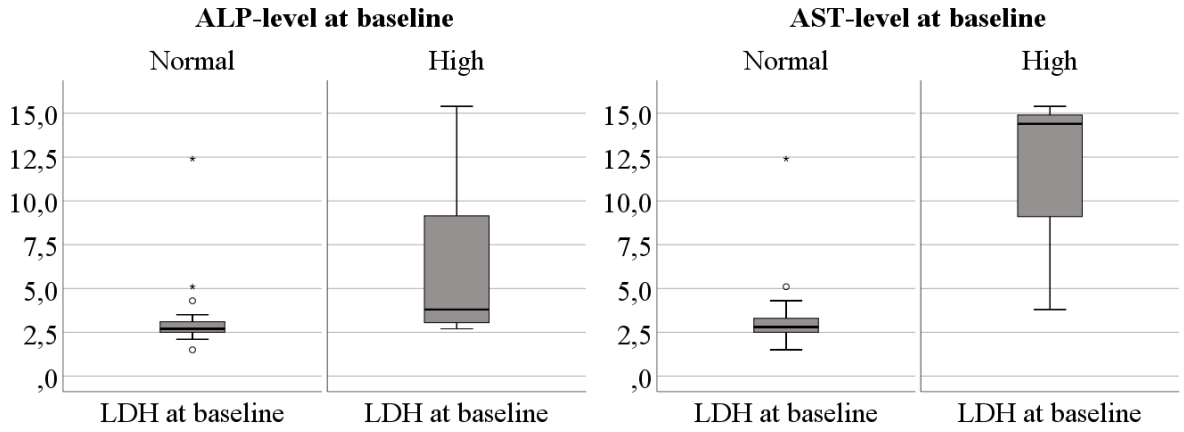

Figure 8. LDH levels in patients with elevated vs normal ALP and AST-levels respectively.

Table 5, LDH levels in patients with normal and high levels of AST and ALP respectively			Count	Median	Maximum	Minimum	Percentile 25	Percentile 75	p-value
LDH at baseline	ALP-level at baseline	Normal	23	2.7	12.4	1.5	2.5	3.1	**0.012**
		High	9	3.8	15.4	2.7	3.1	9.2	
	AST-level at baseline	Normal	28	2.8	12.4	1.5	2.5	3.3	**0.013**
		High	5	14.4	15.4	3.8	3.8	15.4	

Significant values are marked in bold.

6.4. Albumin

Thirty patients had baseline albumin. The mean value of albumin was 40 g/L, and it varied between 28 and 50. Baseline albumin was significantly reduced in patients with ECOG >1 compared to patients with ECOG 0-1 (**Table 6**). No other clinical parameter was significantly associated to reduced levels of albumin. Albumin was positively correlated to creatinine, correlation coefficient 0.461 (p = 0.01, **Table 7**). Albumin was not correlated to age, AST, ALT, ALP or LDH (**Table 7**).

Table 6, baseline albumin levels according to subgroups			Count	Mean	Percentile 25	Percentile 75	Minimum	Maximum	p-value
Albumin at baseline	Treatment response	Responder	9	41	37	45	28	50	0.779
		Non-responder	24	40	39	42	29	48	
	Sex	Female	13	38	35	42	29	44	0.058
		Male	20	42	40	46	28	50	
	Age	<70	28	41	39	44	28	50	0.625
		>70	5	39	35	42	31	48	
	Type of sarcoma	Soft Tissue Sarcoma	23	41	39	43	28	50	0.809
		Bone sarcoma	10	40	34	45	29	46	
	Disease stage	Metastatic disease	19	39	34	45	28	50	0.061
		Localised disease	14	42	41	44	40	46	
	ECOG PS	0-1	17	43	40	45	37	50	**0.01**
		>1	5	37	34	42	31	42	
	Lung metastases	No	23	42	40	44	35	50	0.087
		Yes	10	37	31	45	28	48	
	Bone metastases	No	29	41	37	44	28	50	0.233
		Yes	4	37	29	42	29	42	
	Metastases in abdomen	No	28	40	37	44	28	48	0.709
		Yes	5	41	39	42	35	50	
	Other metastases	No	26	41	40	44	28	50	0.307
		Yes	7	38	37	40	29	48	

Significant values are marked in bold, underlined data are shown in boxplots below (**Figure 9**).

Table 7, Spearman's correlation for albumin at baseline according to linear variables		
Factor	Spearman's correlation coefficient (r_s)	p-value
Age	-0.075	0.692
ALT	-0.023	0.904
AST	-0.282	0.131
ALP	-0.108	0.577
Creatinine	0.461	**0.010**
LDH	-0.028	0.890

Significant values are marked in bold.

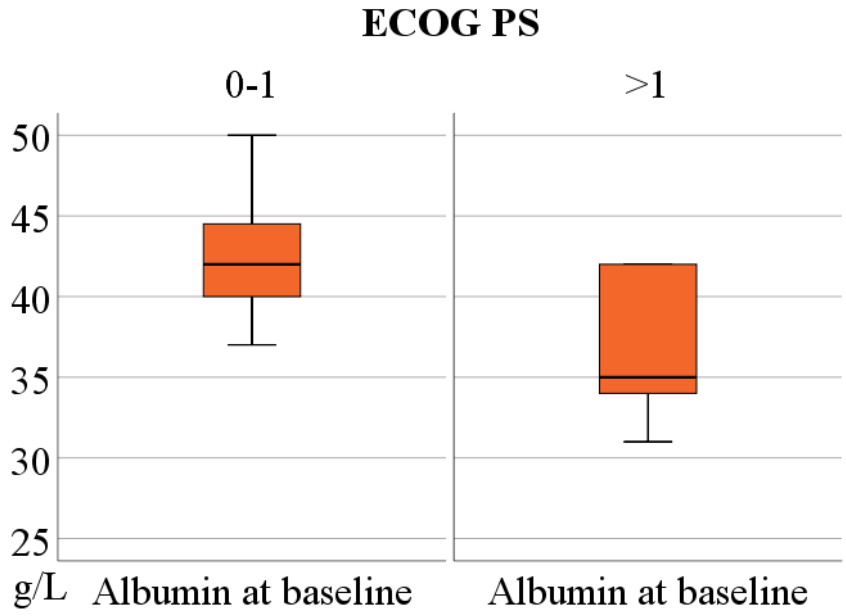

Figure 9. Albumin levels in patients divided by ECOG-levels.

6.5. Monitoring of patients during treatment using LDH and Albumin

LDH and albumin levels were monitored over time in four patients. These patients were chosen since they mirrored different outcomes.

Patient one was a 19-year-old man with a large Ewing sarcoma in the lower abdomen. He started chemotherapy, but the tumour progressed; he then received radiation therapy and subsequently started second line chemotherapy. At last, he received palliative radiation therapy twice, but the disease gradually progressed. The patient passed away approximately six months after initial diagnosis (**Figure 10**). Patient two is a man who at time of diagnosis was 24-year-old with a large Synovial sarcoma in proximity to the liver. The patient received neoadjuvant chemotherapy prior to surgical excision of the tumour. After surgery he received adjuvant chemotherapy and radiation therapy with subsequent oral chemotherapy. The patient

is currently alive and has so far been free from relapse (**Figure 11**). Neither patient one nor patient two had any comorbidities. Because of the significant correlation between LDH and AST, we compared these levels over time in patient one and two (**Figure 12** and **13**). These two curves matched each other well in patient one but not as well in patient two.

Patient three was an 84-year-old woman with angiosarcoma on her right shoulder. She was treated with surgery but had a quick disease relapse. She was then treated with additional surgery with a thoracoscapular amputation. Unfortunately, she was diagnosed with lung metastases and initiated palliative chemotherapy. She had a good treatment response (PR) at first radiologic follow up but had a rapid disease relapse. She received palliative radiation therapy but passed away approximately 10 months after initial diagnosis (**Figure 14**). This patient suffered from several metabolic diseases and was simultaneously receiving treatment for a slow growing metastatic breast cancer. Biopsies of one of the metastases during disease relapse confirmed that it was the angiosarcoma and not the breast cancer that was rapidly progressing. Patient four was a 70-year-old man with metastatic leiomyosarcoma (**Figure 15**). He had a large tumour above the pelvis and many lung metastases. He was initiated on chemotherapy and had a good treatment response. He eventually progressed and was switched to targeted therapy. He remains on this treatment and has not suffered any relapse. In addition to his metastatic sarcoma, he was not treated for any other disease than glaucoma.

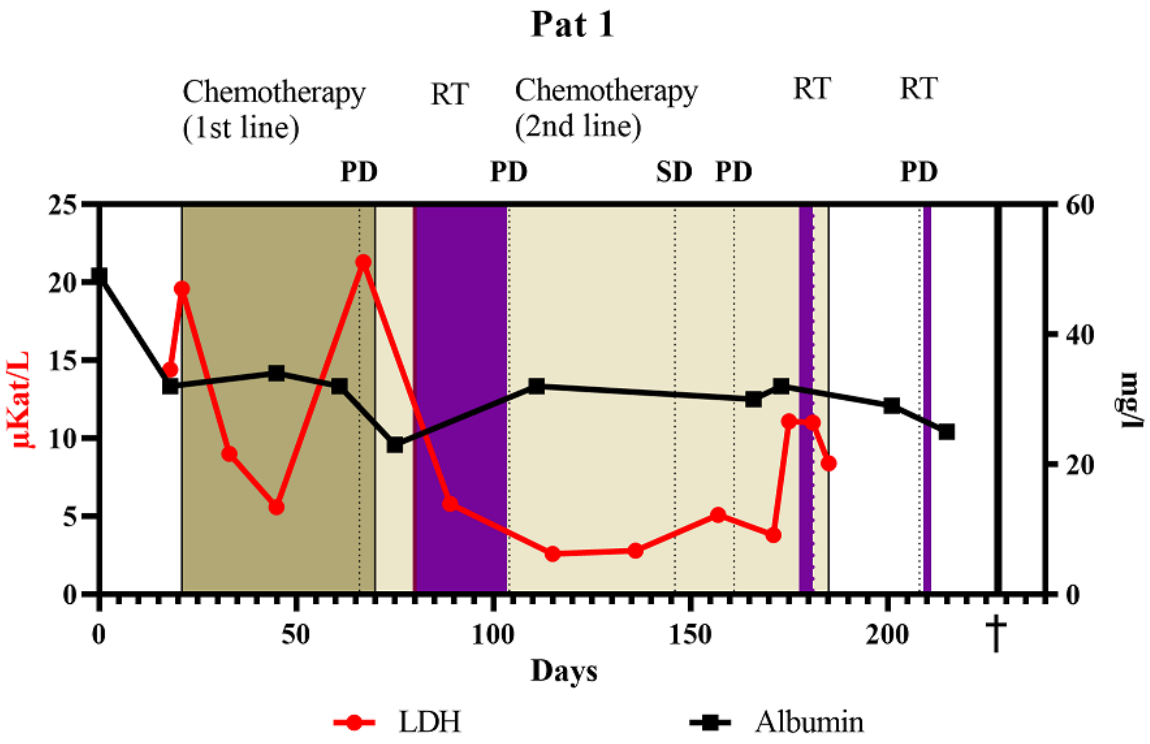

Figure 10. LDH and albumin levels over time in patient 1.
RT = Radiotherapy. PD = Progressive Disease. SD = Stable Disease. † = death.

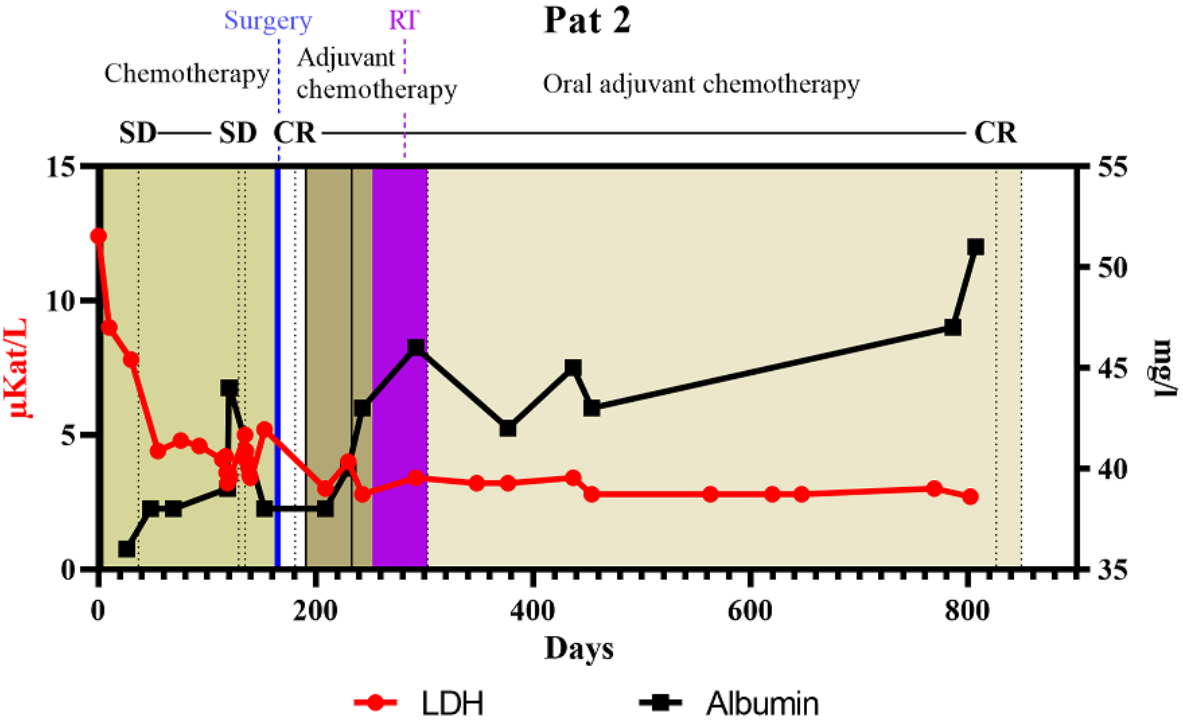

Figure 11. LDH and albumin levels over time in patient 2.
RT = Radiotherapy. SD = Stable Disease. CR = Complete Response.

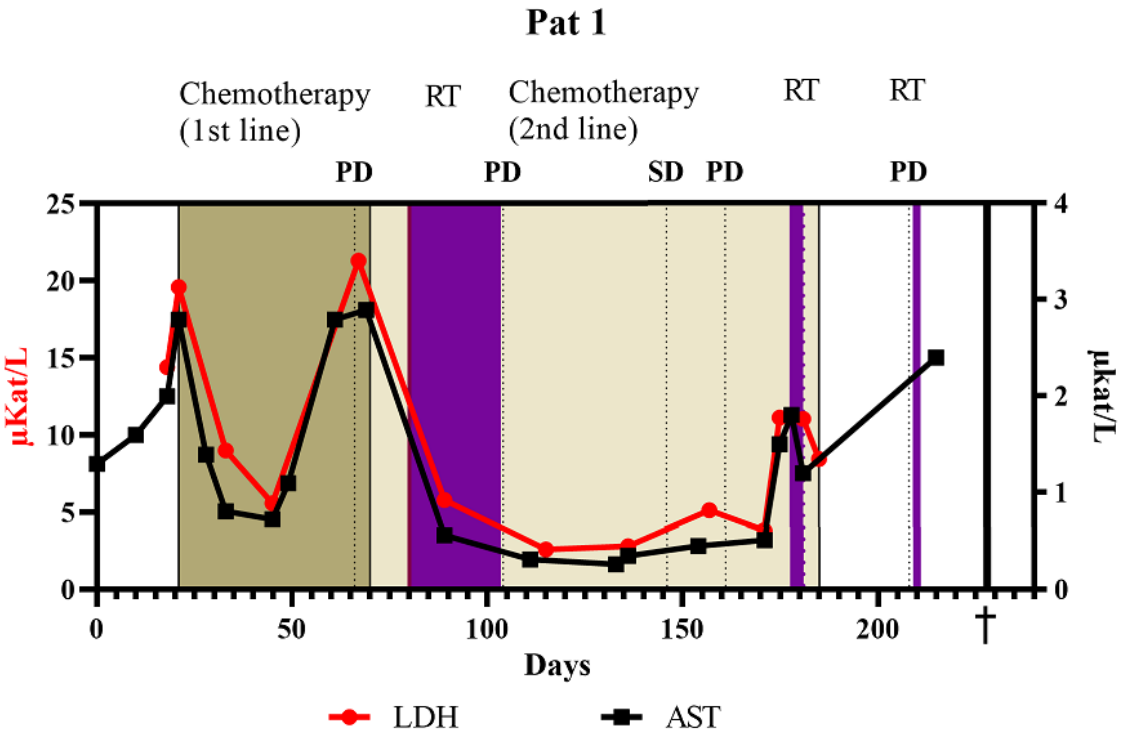

Figure 12. LDH and AST levels over time in patient 1.
RT = Radiotherapy. PD = Progressive Disease. SD = Stable Disease. † = death.

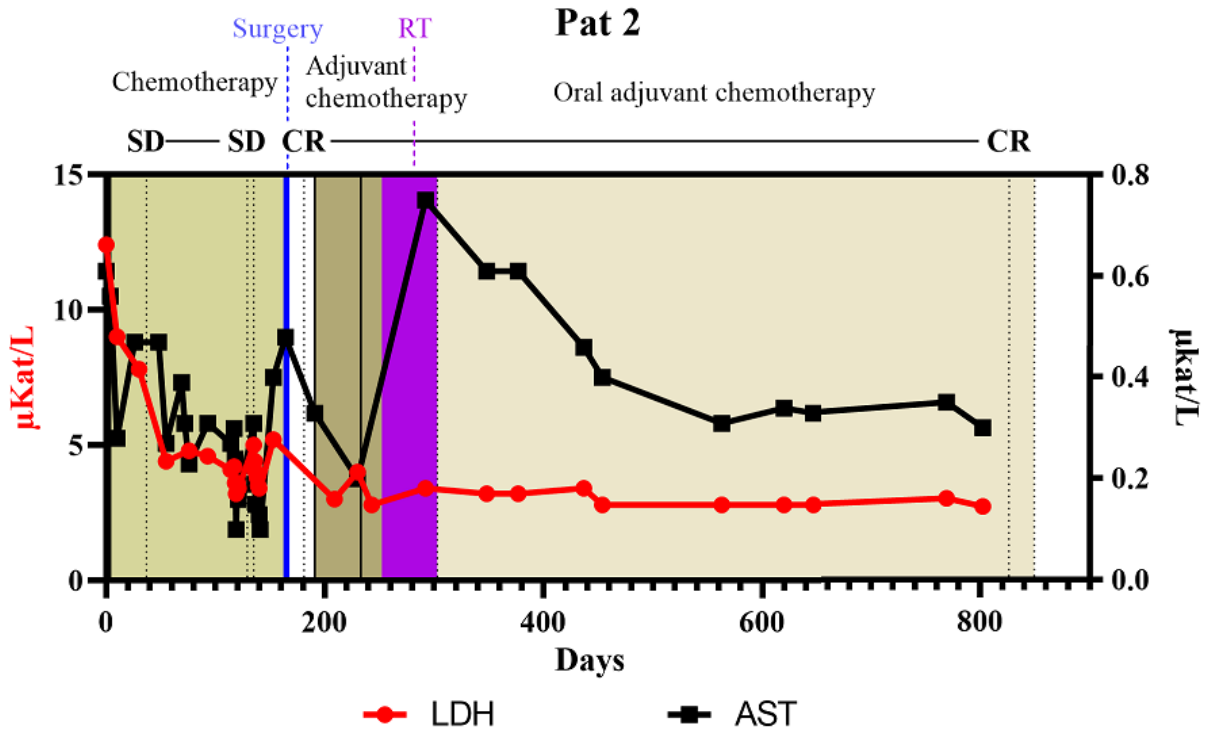

Figure 13. LDH and AST levels over time in patient 2.
RT = Radiotherapy. SD = Stable Disease. CR = Complete Response.

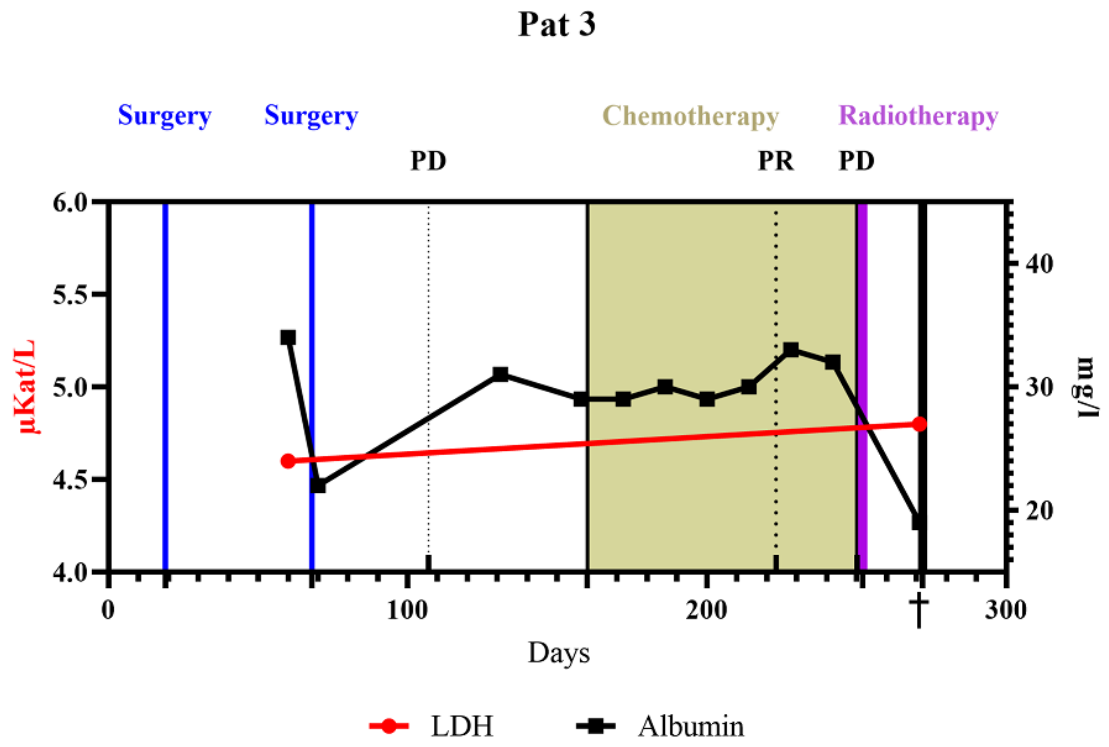

Figure 14. LDH and albumin levels over time in patient 3.
RT = Radiotherapy. PD = Progressive Disease. PR = Partial Response. † = death.

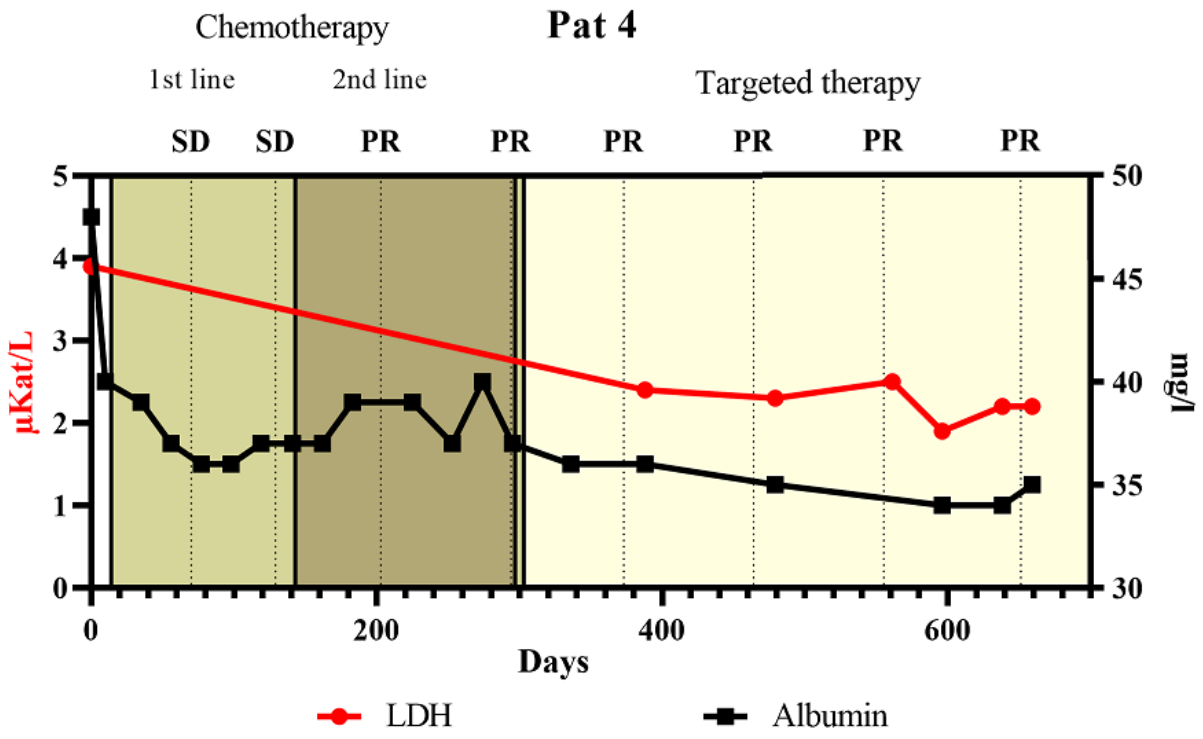

Figure 15. LDH and albumin levels over time in patient 4.
SD = Stable Disease. PR = Partial Response.

7. Discussion

The primary aim of this study was to investigate whether baseline LDH and albumin levels had a predictive value for treatment response at first radiology evaluation. Additionally, we aimed to explore if other factors could explain variances in these tests prior to treatment and if the levels of LDH and albumin differed over time. The result showed no significant difference in baseline LDH and albumin levels regarding treatment response. However, we found differences in baseline levels of LDH and albumin according to clinical and patient related factors. We also observed differences in these markers over time. In some cases, the markers correlated with clinical outcome.

To our knowledge, no other study has been conducted on the value of LDH and albumin as predictors of treatment response. Other studies have instead focused on the markers' values as prognostic factors for survival, which might be a more proper use of these biomarkers.

Both LDH and albumin are markers that can be affected by a variety of conditions, and therefore their sensitivity is low. This might explain why we found no significant differences between responders and non-responders. In addition, our study measured total LDH levels. However, in cancer disease, LDH A is known to be more dominant (35). Therefore, our result might have differed if we had measured the levels of isoenzymes instead of total levels, but this is not done in clinical routine.

Nonetheless, albumin and LDH levels have been shown to be affected in patients with sarcomas and therefore one might suspect that their levels would rise or decrease as a result of treatment. For example, Aparicio et al (1998) found a significant correlation between tumour diameter and LDH levels (37). In addition, Willeger et al (2017) showed that patients with metastatic disease were more likely to have lower albumin levels compared to patients with localised disease (20). In our study cohort, most patients had metastatic disease in both the responder and non-responder group. This might explain why there was no difference in

albumin levels between the two groups. There were also few responders in our study, which might also explain the results.

In contrast to other studies, where cut off levels for LDH were determined based on the levels in the study population, we used the reference values given from the laboratory. Even if the calculated levels might demonstrate a more proper cut off for a specific outcome, this is also an arbitrary level since it will vary between different cohorts and repeated studies must be undertaken before any cut off point can be incorporated into clinical praxis. In contrast, studies focusing on albumin levels more often used accepted reference values, like we did in our study. For example, Nakamura et al who used the World Health Organization's (WHO) definition of albumin levels (19). However, their aim was to evaluate albumin as a prognostic factor. Thus, our result together with prior findings indicates that albumin might be more useful as a prognostic marker for survival than a predictive marker for treatment response.

Another more proper use of these markers could be to include them in an index. The prognostic value of including albumin in an index have been shown in previous studies (50). Therefore, to evaluate these markers as part of an index or simply the two markers together to predict treatment outcome might have revealed another significance.

Moderate positive correlations were found between LDH and AST, and LDH and ALP but not LDH and ALT. Elevated LDH indicates tissue damage, from any organ in the body including liver damage (35). AST is, together with ALT, regarded as an indicator for liver damage. However, ALT is considered more specific for liver damage, whereas AST is more unspecific and is also found in other tissues such as cardiac and skeletal muscles (51). Therefore, the correlation found in this study between LDH, and AST can reflect liver damage as well as other damages that leads to cell destruction.

In prior studies ALP has been shown to be higher in patients with osteosarcomas (22) and to predict presence of skeletal metastases (52). Furthermore, ALP levels have been shown to correlate to LDH levels in osteosarcoma (39). This suggests that the correlation between LDH and ALP that we observed could be of biological significance.

When comparing patients with high respectively normal AST and ALP levels to their LDH levels we found a significant difference in LDH levels between patients with high levels of these markers compared to the group with normal levels. This supports our correlations discussed above. However, it is important to highlight that this was an explorative analysis.

There was an association between elevated LDH levels and lung metastases, however no difference was found between patients with metastatic and localised disease. Prior studies have found a correlation between LDH and skeletal metastases, but not with lung metastases. However, the correlation with skeletal metastases disappeared when they corrected for tumour volume (52). Our analysis did not include tumour volume and therefore, the elevated LDH levels we saw in patients with lung metastases might be a result of a higher tumour volume and not the lung metastases themselves. Except for lung metastases, the metastases subgroups contained too few patients for analysis.

In the observation of LDH levels over time we saw two different outcomes. In the first patient, the LDH levels fluctuated during treatment until his death, despite tumour progression. The second patient's LDH generally decreased during treatment and then stagnated after surgery, which might illustrate a case were the LDH level mirrored the patient's tumour burden and treatment response. The reasons for the fluctuations of LDH levels in patient one are unclear. Since LDH measures tissue damage, LDH levels could be expected to increase after treatment or after tumour progression. However, the LDH fluctuations were not correlated to these factors. Alternatively, elevated LDH levels might

suggest that the tumour exerted pressure over surrounding tissues. Nonetheless, the LDH levels might then be expected to be consistently high and not fluctuating.

Interestingly, when comparing LDH and AST levels over time, these two curves matched each other well in patient one but not as well in patient two. Therefore, this might reflect two different causes of LDH elevation in the patients. Whether the LDH and AST correlation in patient one is due to liver damage or any other tissue damage is unclear.

An important remark of our LDH results is that three included patients appeared to be outliers (**Figure 2**). Since the study cohort was very small, three outliers can highly impact the result and the interpretations of it. However, they also mirror the actual clinical situation. Two of these patients had metastatic disease (one of these patients are illustrated in **Figure 10** and **12**), while the other had a localised sarcoma (**Figure 11** and **13**). The elevated LDH levels in the patients with metastatic disease could be attributed to a higher tumour burden due to metastases. What the elevated LDH level stands for in the patient with Synovial sarcoma, is intriguing. Bacci et al (2004) have shown that a higher LDH correlates to shorter time to relapse (39). This could imply that this patient has a higher relapse risk and therefore should be followed up more extensively with radiologic evaluation. Yet, this is only a speculation and needs to be properly investigated in future studies.

We found a positive correlation between albumin and creatinine. What this stand for is unclear. It could be explained as patients suffering from dehydration leading to higher concentrations of measured markers in their blood. However, except for this, we find no other obvious biological explanation for this correlation. Therefore, it can also be regarded as a type I-error (false positive) due to coincidence. Altogether it needs to be further investigated before any conclusions can be drawn. Further, albumin was correlated to ECOG PS. A higher ECOG PS are a known factor for poorer prognosis (14, 21, 25). Hypoalbuminemia is also a known factor for poorer prognosis (42). Thus, this result is consistent with earlier research.

When we observed albumin levels over time, the albumin levels in patient three illustrated a typical example of when albumin correlates to a poorer prognosis. The patient had normal albumin levels at the time of diagnosis, that then decreased when her disease progressed. In the last weeks before death the albumin levels sank considerably. However, even though patient four responded to treatment, his albumin levels have decreased the last year. This might be due to the therapy he receives, or it another unrelated condition that we are not aware of.

To return to the analysis of responders and non-responders, only nine patients were responders, while the majority (24 patients) were non-responders. This might be due to several reasons; small study cohort, inclusion criteria, follow up time, inclusion of several subtypes, and method for treatment evaluation. The short follow up time for this study might have led to patients not receiving treatment for long enough time to reach response criteria, thus leading to too few responders. Furthermore, our study cohort included many subtypes of sarcomas that are known to be poor responders to chemotherapy, for example myxofibrosarcoma and dedifferentiated liposarcoma, which might have affected the outcome (12). Moreover, we included patients with stable disease in the non-responder group, which can be considered as a harsh division. Additionally, a study evaluating early and late tumour volume response to treatment in correlation to survival, observed no effect on survival of an early tumour volume response (within 18 weeks from start of treatment) (29). It might therefore be that the way we measured treatment outcome is an inadequate way of evaluating tumour volume response, which might conceal proper differences between the groups.

Finally, compared with other studies on sarcoma patients with small cohorts, this study cohort is quite similar regarding size and sex (19, 43). However, we only had seven patients over the age of 70, a lower median age and included both soft tissue and bone sarcomas. Six patients had to be excluded because their radiology prior to treatment exceeded six weeks. Seven

patients had only one of the laboratory tests measured before start of treatment. This is explained by the patients in this study being followed up according to clinical routine and not according to any study protocol. However, this is not optimal since it makes it harder to evaluate the treatment response in a correct way.

7.1. Methodological considerations

The main strength of this study is its study design. The study was conducted as a retrospective, observational study, which minimised the risk for bias in the monitoring of patients. At the same time, we had no control over sample collection and how they were monitored and analysed. Even so, this is a valid method for these study aims and has been used in several similar studies prior to this and it also closer reflects the real-life data of the clinic. We also performed a univariate explorative analysis, were we searched for differences. Using this method might lead to findings of false associations. However, it is the best available method when investigating this research aim in this kind of data set. Even so, the correlations we found need to be validated in other studies (with larger data sets and be compared with a control group) before any conclusions can be drawn.

Another strength is that it mirrors the population of sarcoma patients in the Region of Västra Götaland. All patients that receive oncologic treatment for sarcoma at the Oncology Department at Sahlgrenska University Hospital are asked for inclusion in the sarcoma test study. This makes the population quite complete. However, it might bypass patients that only receive surgical treatment (though this is not a problem for this particular study), and it might be a bias that some patients are not questioned or decline participation. Moreover, we do not know how many patients declined the offer. There is also a risk that the study population suffers from under coverage. Therefore, if all patients receiving oncologic treatment at Sahlgrenska University Hospital were included, this would have improved the study.

All patients included in the study were diagnosed, treated, and monitored during the last 10 years. This provides strength to the study since all patients are handled similarly. It is also likely that the diagnostic process is comparable since diagnosis classifications have not changed since 2013 (7).

Despite the strength that the population mirrors the sarcoma population in the region, this provides a problem for the analysis. The patient group is heterogenous in subtypes, age, and type of treatment, which might conceal true differences between subgroups. This is a recurrent problem for research in the sarcoma area. Studies are either performed on small homogenous populations or include patients over several decades. This is problematic since it might lead to problems with sample size or introduce bias since patients are handled differently over time. One might argue that it would be optimal with large cohorts, while retaining the heterogeneity by including different subgroups.

Another limitation of this study is how the radiologic and histologic examinations were done. Ideally, these examinations would have been carried out in unison by the same examinator, or a team of examinators, to provide a more accurate result. However, this was not possible within the limitations of the master project.

Additionally, this study has several possible confounding factors. As stated earlier, these include sample handling and processing, inclusion of patients and sarcoma subtypes. Other possible confounders are comorbidities, type of metastases, and that we excluded patients with successful surgery. Even though we did not control the analyses for comorbidities, we did correlate the result to creatinine and liver values as a mean to correct for this. That patients with successful surgery were excluded is a confounding factor since it might imply that the included group from the beginning are less likely to respond to treatment. However, it would not be possible to measure treatment response in patients with radical surgery if they

did not suffer from relapse. These patients are also handled by another department, that do not take LDH and albumin tests in clinical routine.

At last, the assumptions were met for the statistical tests that were used. Ideally, if the study population had included more individuals, we could have had performed logistic regression analysis which would have given us a more proper understanding of factors that might affect the treatment response.

8. Conclusions

In conclusion, we saw no difference in baseline LDH and albumin levels between treatment response in the studied patient cohort. However, the study population was limited and heterogenous. Therefore, more studies are needed before these markers can be dismissed as predictive markers for treatment response.

In addition, associations were observed between clinical parameters and LDH and albumin levels. The LDH variances we saw could be associated with total tumour burden and type of sarcomas, these results imply that there might be an association between LDH levels and tumour volume and sarcoma subtype, respectively. However, LDH levels were also correlated to AST and ALP levels which indicates that it is an unspecific marker. Therefore, these correlations need to be further investigated before any conclusions about causality can be drawn. Associations were also observed between lower albumin levels and poorer ECOG PS. Higher ECOG PS indicate poorer prognosis. Equally, albumin is a known predictor of poor prognosis in cancer patients. Therefore, this result was in line with prior research.

We believe that LDH and albumin might be useful markers together with novel biomarkers for sarcoma patients in the future. For example, the value of albumin as part of an index to indicate prognosis, have been shown in earlier studies. Therefore, we propose that future research should focus on these markers as part of indexes to predict treatment response, instead of focusing on these markers individually. Hopefully, further research will bring better tools for the clinician to use when handling sarcoma patients and help improve the outcome and monitoring of this patient population.

9. Populärvetenskaplig sammanfattning

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Laktat-dehydrogenas och albumin som markörer för att förutsäga behandlingsresultat hos sarkompatienter

I den här studien undersökte vi förekomsten av två markörer som man kan hitta i blodet: laktat-dehydrogenas (LD) och albumin. Vi ville undersöka om dessa kunde användas för att förutsäga behandlingsresultat hos patienter med sarkom, eftersom det i nuläget saknas bra metoder för detta.

Sarkom är ett samlingsnamn för över 70 olika cancerdiagnoser i skelett- och mjukdelar. Det är en ovanlig cancergrupp som utgör mindre än 1% av all cancer i Sverige. 5-årsöverlevnaden är ca 65%, vilket är lägre än för många vanligare cancersjukdomar. Sjukdomen drabbar alla åldrar, men blir vanligare i högre åldrar. LD är ett protein som finns i kroppens alla celler. Nivån av LD i blodet stiger när celler går sönder. Albumin är också ett protein som finns i blodet. Vid inflammation i kroppen (exempelvis vid cancer) sjunker albuminnivån i blodet. Låga nivåer av albumin och höga nivåer av LD har i tidigare studier visats ge en sämre canceröverlevnad.

Att bättre kunna följa sarkompatienter med hjälp av blodprover skulle förbättra omhändertagandet av patienterna. Detta genom exempelvis snabbare utvärdering av behandlingsresultat, tidigare upptäckt av canceråterfall och avgöra när det är lämpligt att röntga en patient för att använda vårdens resurser så smart som möjligt. Därför ville vi undersöka om man med hjälp av två vanliga blodprov, som mäts på alla sjukhuslaboratorier i Sverige, kunde få en indikation på om patienten skulle svara på behandling.

Vår studie omfattade 33 individer som alla hade gett sitt samtycke till att delta. Studiematerialet samlades in genom att vi sökte i patienternas journaler efter specifika faktorer, till exempel hur patienterna skattade sin fysiska aktivitetsnivå innan behandling och behandlingssvar. Studiepopulation delades in i två grupper utifrån huruvida de svarat på behandling eller ej. Detta gjordes via röntgenundersökningar vid första uppföljande röntgen eller vävnadsprover (biopsier) från tumören efter operation. Data illustrerades i grafer och vi använde sedan statistiska metoder för att analysera resultatet och se om en skillnad förelåg mellan grupperna.

Vi kom fram till att dessa markörer inte gav någon fingervisning om huruvida patienten svarar på behandling. Däremot sågs en koppling mellan blodproverna och andra faktorer. Till exempel att LD verkade ha ett samband med förhöjda lever- och skelettprover, skelettsarkom och metastasförekomst. Detta tolkade vi som att provet var ospecifikt, det vill säga att provet kan vara förhöjt av olika orsaker samt att det kan finnas ett möjligt samband mellan LD och tumörvolym. Men för att säkert kunna svara på om detta samband faktiskt finns och hur det kan användas i vården av patienter så måste ytterligare studier göras. Vad gäller albumin, så noterade vi lägre albuminnivåer hos patienter med lägre självrapporterad fysisk aktivitetsnivå. Detta stämmer överens med tidigare forskning.

Den här studien visade inga tydliga samband. Utifrån tidigare studier vet vi att proverna har ett värde för att förutsäga prognos hos cancerpatienter. Förhoppningen är därför att dessa markörer i framtiden kommer att kunna användas tillsammans med nya, mer specifika markörer för att bättre följa sarkompatienter och förbättra deras situation.

10. Acknowledgements

I would like to thank my supervisor Anders Ståhlberg for valuable input and insightful comments during the project and on my written report.

I would also like to thank my co-supervisor Christoffer Vannas for guiding me through this project in a supportive and dedicated way despite many other tasks and commitments. Thanks for your time and patience with all my questions, your help with the analysis, and for valuable input on my written report.

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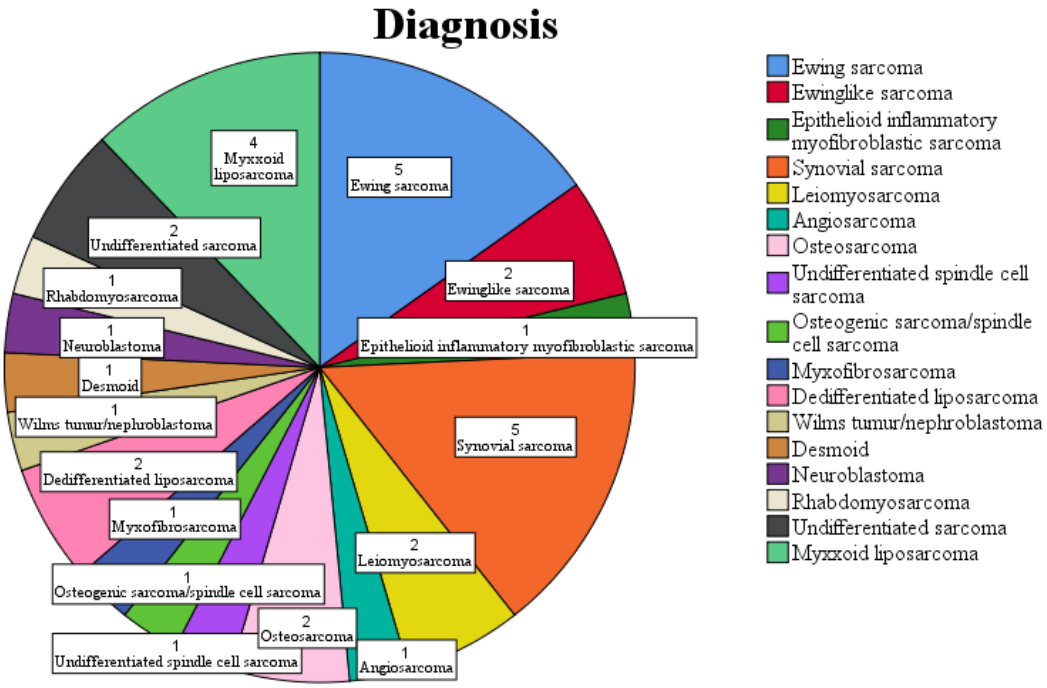
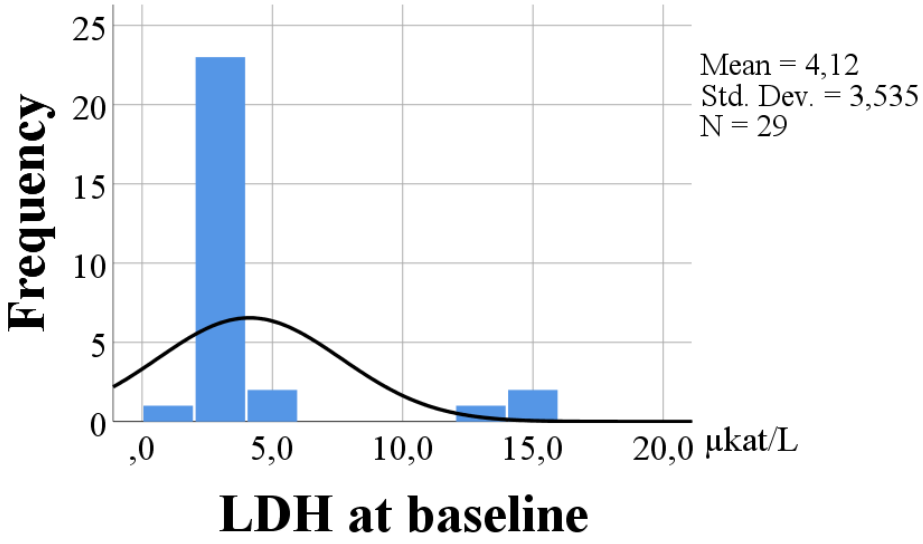
12. Appendix

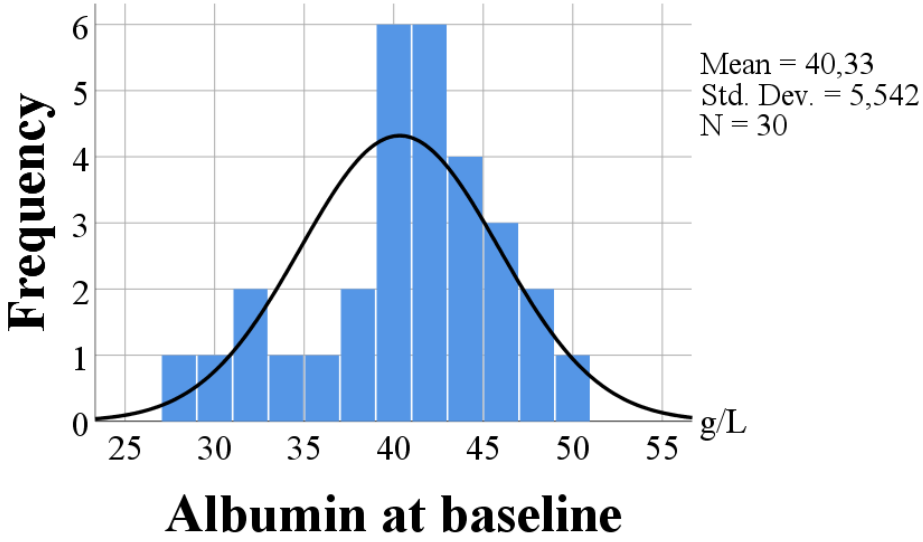
	Appendix table 1. ECOG Performance Status.
Grade	ECOG Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair.
5	Dead.
	Reference: Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol.* 1982;5:649-655.



Appendix figure 1. Diagnoses within study population.



Appendix figure 2. Distribution of LDH at baseline.



Appendix figure 3. Distribution of albumin at baseline.