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Inflammatory Biomarkers Can Differentiate Acute Lymphoblastic Leukemia with Arthropathy from Juvenile Idiopathic Arthritis Better Than Standard Blood Tests

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Objective To evaluate the predictive value of biomarkers of inflammation like phagocyte-related S100 proteins and a panel of inflammatory cytokines in order to differentiate the child with acute lymphoblastic leukemia (ALL) from juvenile idiopathic arthritis (JIA).

Study design In this cross-sectional study, we measured S100A9, S100A12, and 14 cytokines in serum from children with ALL (n = 150, including 27 with arthropathy) and JIA (n = 236). We constructed predictive models computing areas under the curve (AUC) as well as predicted probabilities in order to differentiate ALL from JIA. Logistic regression was used for predictions of ALL risk, considering the markers as the respective exposures. We performed internal validation using repeated 10-fold cross-validation and recalibration, adjusted for age.

Results In ALL, the levels of S100A9, S100A12, interleukin (IL)-1 beta, IL-4, IL-13, IL-17, matrix metalloproteinase-3, and myeloperoxidase were low compared with JIA ($P < .001$). IL-13 had an AUC of 100% (95% CI 100%-100%) due to no overlap between the serum levels in the 2 groups. Further, IL-4 and S100A9 had high predictive performance with AUCs of 99% (95% CI 97%-100%) and 98% (95% CI 94%-99%), respectively, exceeding both hemoglobin, platelets, C-reactive protein, and erythrocyte sedimentation rate.

Conclusions The biomarkers S100A9, IL-4, and IL-13 might be valuable markers to differentiate ALL from JIA. *(J Pediatr 2023;258:113406)*.

Acute lymphoblastic leukemia (ALL) may be misdiagnosed as juvenile idiopathic arthritis (JIA) since a significant proportion of childhood leukemia presents with arthropathy often combined with few and less robust classical signs of leukemia.[1-3] ALL is the most common childhood neoplasia, with an approximate annual incidence of 40 per million in the Nordic countries.[4] Further, ALL is the type of malignant neoplasm most frequent associated with arthropathy (arthralgia and arthritis) at disease onset.[5-9] JIA is the most common chronic inflammatory joint disease in children, with an annual incidence of 15 per 100 000 children in the Nordic countries.[10,11] ALL with arthropathy is at high risk of being misdiagnosed as JIA with the risk of diagnostic delay.[1,12,13] Routine inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) have obvious limitations to differentiate ALL from JIA.[3,14] Thus, in demand are sensitive and specific biomarkers that can be used to optimize the diagnostic process.

The phagocyte-related and proinflammatory S100A8/A9 and S100A12 proteins have been identified as important markers of inflammation in several conditions, including inflammatory arthritis.[14-16] S100A9 forms a heterocomplex with S100A8 (also called MRP8/14) and can be detected by monoclonal S100A9 antibodies as used in immunoassays for its measurement in feces or

ALL	Acute lymphoblastic leukemia	JIA	Juvenile idiopathic arthritis
AUC	Area under the receiver operating characteristic curve	CCL-2	Chemokine ligand 2
		CRP	C-reactive protein
		ESR	Erythrocyte sedimentation rate
CD25	Interleukin-2 receptor alpha chain	IL	Interleukin
JADAS71 score	Juvenile Arthritis Disease Activity Score for 71 joints	MMP-3	Matrix metalloproteinase-3
		MPO	Myeloperoxidase
		WBC	White blood cell

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blood. Several studies have investigated S100A8/9 proteins in JIA and revealed a correlation with disease activity.[14,15,17-24] In ALL, the evaluation of S100 proteins is very limited.[22,25] Cytokines are effector molecules for cells involved in the immune and inflammatory responses and the hematopoietic cell proliferation and differentiation.[26-29] In JIA, tumor necrosis factor-alpha, interleukins (ILs) IL-6, IL-10, IL-12, and IL-18 have been shown to correlate with disease activity.[30-38] IL-4 and IL-13 are critical components of Th2-cell–mediated immunity and recently have been described as important markers in the pathogenesis of inflammatory arthritis.[39,40] In rheumatoid arthritis, IL-4 and IL-13 have been proven to downregulate the inflammatory processes and correlate with disease activity.[41-44] In ALL, cytokines have been investigated only to a minimal extent in vitro and in small cohorts, mainly in adults.[25,45-49]

There exist 2 different clinical scenarios for physicians to consider regarding differentiating ALL and JIA. The first is the child with systemic JIA with the risk of being misdiagnosed as several systemic inflammatory diseases, such as systemic infections, macrophage-activating syndrome, and various types of cancers, one of them being ALL. This scenario has been the main focus of the literature, revealing S100A9, S100A12, and IL-18 as valuable biomarkers identifying systemic JIA.[14,22,25] The other scenario, which is the focus of this paper, is the child with ALL mistaken for nonsystemic JIA, which may delay ALL diagnosis.[3,14] Only a few studies have evaluated newer inflammatory biomarkers in order to optimize the diagnostic process and avoid ALL with arthropathy being misdiagnosed as JIA.[50] The primary aim of our study was to investigate the predictive value of biomarkers of inflammation to differentiate ALL from JIA in pediatric patients.

Methods

We included consecutive cases of newly diagnosed patients with JIA from defined geographical areas of Denmark, Norway, and Sweden, as previously described in detail.[51,52] They had disease onset from January 1, 1997, to June 30, 2000. The Finnish portion of the original cohort had no access to storage of blood samples at baseline, and, accordingly, this center was not included in the present study. For the JIA cohort, the data were collected 6 months (–1/+ 2 months) after disease onset. The JIA serum samples were 1:4 prediluted in tris-buffered saline.

We further included consecutive, newly diagnosed children with ALL from a nonselected, population-based Danish ALL cohort, with inclusion from January 1, 2001, to December 31, 2018, from Aalborg and Aarhus University Hospitals, Denmark. For the ALL cohort, data were collected as close to diagnosis as possible (+/– 1 week) and before anticancer therapy was initiated. All children included in the study had an established diagnosis of ALL or JIA at the time of inclusion. The diagnosis of ALL was based on bone marrow biopsy and the diagnosis of JIA according to the International League of Associations for Rheumatology criteria for JIA.[53] Arthralgia was defined as pain distinctly localized to 1 or more joints. Arthritis was defined as swelling within a joint and/or limitation of joint motion with joint pain. Arthropathy was defined as arthralgia and/or arthritis.

The following data were collected in both cohorts: demographics (age, sex), clinical presentation (disease category, risk group, number of active and cumulative joints with arthritis), and clinical laboratory variables (hemoglobin, platelets, ESR, CRP). For the children with ALL, we also collected counts of white blood cells (WBCs), neutrophils, monocytes, lymphocytes, and blasts in peripheral blood. For the children with JIA, the Juvenile Arthritis Disease Activity Score for 71 joints (JADAS71 score) was used to evaluate disease activity.[54]

Assays

Serum samples for biomarker analysis were stored at –80°C. The serum concentrations of S100A9 (as a measurement of the S100A8/A9 complex), S100A12, IL-1β, IL-4, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-18, tumor necrosis factor-alpha, matrix metalloproteinase 3 (MMP-3) and myeloperoxidase (MPO), chemokine ligand 2 (CCL-2), and soluble CD25 (CD25) were determined by multiplexed bead array assay according to the manufacturer's instructions (R&D Systems). Data acquisition and analysis were performed on a MAGPIX instrument (Merck Millipore) using xPONENT v4.2 software (Luminex). The analyzing laboratory in Muenster, Germany, was blinded to the patient's characteristics.

Statistics

The concentrations of the biomarkers were non-normally distributed, as evaluated by QQ-plot and histograms. Therefore, the results were presented as median with first-third IQR and compared using the Mann–Whitney *U* test for continuous data and Fisher exact test for dichotomous variables. In addition, Spearman rank-order correlation was used to evaluate the correlation between the biomarkers, the laboratory values, and age.

In order to establish prediction models for ALL detection, we used logistic regression with fractional polynomials to handle nonlinearity in regressions. First, we performed univariate screening of potential predictors according to the outcome variable (ALL). For the best markers, we constructed logistic regression models, as we performed internal validation using repeated 10-fold cross-validation and recalibration, adjusted for age.[55] The performance of the models was evaluated using the area under the receiver operating characteristic curve (AUC), sensitivity, specificity, positive predictive values, negative predictive values, and accuracy using a threshold of 0.5 for the predicted probabilities of ALL. Further, for the best markers, we identified optimal cut-offs from receiver operating characteristic analyses with the goal of a sensitivity of minimum 95% due to the severity of ALL.

We used Stata, version 17.0 (StataCorp) for all statistical analyses performed together with a professional statistician. Statistical significance was established at 5% for a 2-dimensional test.

Ethics

The study was approved by the Medical Ethics Committee (1-10-72-206-19) and the Danish Data Protection Agency (1-16-02-214-16). The data and samples of the JIA cohort were further approved by the national research committees (1-10-72-280-13, 2012/2051, Dnr 2014/413-31, 174/13/03/03/2014), and all patients gave their written informed consent. For patients with ALL, serum samples were collected from the Nordic Society of Pediatric Oncology and Hematology biobank in Uppsala, and all patients gave their written informed consent.

Results

Of 257 consecutive children diagnosed with ALL from 2001 to 2018, serum was available for biomarker analysis in 150 of 257 (58%) participants, who were included in the present study. The age at onset and subgroup distribution did not differ for the included group compared with those with no available serum. The ALL cohort included 123 of 150 (85%) pre-B cell ALL and 22 of 150 (15%) T-cell ALL. Arthropathy at presentation occurred in 18% (27/150), including 20/150 (13%) with arthralgia, and 7 of 150 (5%) with arthritis. The median number of joints involved was 2 (IQR 1-7, range 1-9). Arthropathy mainly occurred in the pre-B ALL group, present in 20% (26/128), whereas only 1 patient with T-cell ALL presented with arthralgia. In the group with T-cell ALL, we found higher levels of hemoglobin, platelets, neutrophils, S100A9, S100A12, and MPO, compared with the group with pre-B ALL. In contrast, the level of CCL-2 was lower in the group with T-cell ALL (**Table I**). Due to the risk of inhomogeneous results, we excluded the children with T-cell ALL from the analysis when comparing $ALL_{arthropathy}$ (n = 26) with the children without arthropathy (n = 102). The levels of hemoglobin, platelets, neutrophils, CRP, S100A9, S100A12, and MPO were higher in the group with $ALL_{arthropathy}$ compared with the patients without any arthropathy. In contrast, the levels of IL-10 were lower in the patients with pre-B $ALL_{arthropathy}$ compared with the patients without any arthropathy (**Table II**).

Of 510 consecutive children diagnosed with JIA from 1997 to 2000, serum for the biomarker analysis was available in 236 (46%), who were included in the present study. Age at onset, JIA categories, the number of active joints, or JADAS71 score did not differ for the included group compared with those with no available serum. The distribution of JIA categories was oligoarthritis 119 of 236 (50%), polyarticular rheumatoid factor-negative JIA 46 of 236 (19%), polyarticular rheumatoid factor-positive JIA 5 of 236 (2%), systemic JIA 11 of 236 (5%), enthesitis-related arthritis 219 of 236 (8%), juvenile psoriatic arthritis 2 of 236 (1%), and undifferentiated JIA 34 of 236 (14%). The median number of active joints was 1 (IQR 0-3, range 0-31), and the median number of cumulative joints was 3 (IQR 1-7, range 1-45). The JADAS71 score was 5 (IQR 2-11, range 0-43).

Table I. Comparison of the protein levels for children with T-ALL vs pre–B-ALL

Values	T-ALL n = 22	Pre–B-ALL n = 128	*P* value
Hemoglobin, g/dL	10 (8-12)	7.4 (5.3-9.5)	<.001
Platelets, × 10^9/L	58 (41-157)	43 (16-101)	.16
WBC, × 10^9/L	22 (5-86)	9 (4-41)	.09
Neutrophils, × 10^9/L	2.1 (0.4-4.9)	0.4 (0.2-1.0)	<.001
Monocytes, × 10^9/L	0.5 (0.2-2.0)	0.3 (0.1-1.1)	.16
Lymphocytes, × 10^9/L	4.6 (1.9-19)	5.6 (3.5-15)	.76
CRP, mg/L	14 (8-36)	21 (7-51)	.35
ESR, mm/H	47 (19-104)	68 (39-104)	.35
S100A9, pg/mL	124 (45-288)	40 (21-85)	<.001
S100A12, pg/mL	479 (221-1234)	78 (28-250)	<.001
TNF-alpha, pg/mL	11 (4-20)	7.6 (4.6-14.2)	.37
IL-1 beta, pg/mL	1.3 (1.2-1.9)	1.3 (1.1-2.3)	.74
IL-4, pg/mL	11 (9-15)	13 (10-16)	.08
IL-6, pg/mL	2.4 (1.5-3.4)	3.1(1.8-6.3)	.13
IL-10, pg/mL	4.5 (1.0-11.5)	4.1 (1.8-9.8)	.91
IL-12p70, pg/mL	15 (7-23)	20 (10-26)	.28
IL-13, pg/mL	90 (79-97)	90 (79-97)	.60
IL-17, pg/mL	2.0 (0.2-2.0)	1.7 (1.1-2.0)	.75
IL-18, pg/mL	362 (138-618)	329 (218-557)	.71
MMP-3, pg/mL	1386 (1053-1998)	1360 (970-2553)	.92
MPO pg/mL	2450 (976-4361)	451 (187-1197)	<.001
CCL-2, pg/mL	276 (163-401)	359 (246-554)	.03
CD25, pg/mL	1000 (713-1322)	1155 (873-1643)	.16

B-ALL, B-cell acute lymphoblastic leukemia; *CCL-2*, chemokine ligand 2; *CRP*, C-reactive protein; *ESR*, erythrocyte sedimentation rate; *IL*, interleukin; *MMP-3*, matrix metalloproteinase 3; *MPO*, myeloperoxidase; *T-ALL*, T-cell acute lymphoblastic leukemia; *TNF*, tumor necrosis factor; *WBC*, white blood cells.
Values are medians with IQRs.

In **Table III**, the clinical characteristics and the level of the biomarkers are compared for JIA (n = 236) vs ALL (n = 150) and $ALL_{arthropathy}$ (n = 27), respectively. Hemoglobin and platelets were low in both the total ALL cohort and the subgroup with $ALL_{arthropathy}$ compared with JIA, whereas CRP and ESR were greater in JIA than ALL (**Table III**). The levels of S100A9, S100A12, IL-1 beta, IL-4, IL-6, IL-13, IL-17, MMP-3, and MPO were low in ALL and $ALL_{arthropathy}$ compared with JIA. The levels of IL-13 had no overlap between the ALL (range 58-138 pg/mL) and the JIA group (range 256-2881). In contrast, IL-18, CCL-2, and CD25 levels were higher in ALL and $ALL_{arthropathy}$ compared with JIA (**Table III**). The same differences were found when comparing the patients with nonsystemic JIA (n = 225) with those with pre-B ALL (**Table IV**). The 11 patients with systemic JIA had very high levels of S100A9, S100A12, and IL-18. When comparing systemic JIA vs nonsystemic JIA, we found significantly higher levels of S100A9 ($P = .006$), S100A12 ($P = .007$), and IL-18 ($P < .001$).

The associations between the 15 biomarkers and the standard blood tests are illustrated in heat plots for the ALL and JIA cohorts (**Figures 1** and **2**). In ALL, most biomarkers had strong positive correlations to CRP. In contrast, ESR was negatively correlated with S100A9, S100A12, and MPO. S100A9, S100A12, and MPO had strong positive correlations with circulating neutrophils and, to a smaller extent, with WBC and hemoglobin concentrations. The interactions between the biomarkers were evident with strong positive correlations. We highlight high positive

Table II. Clinical characteristics and level of proteins in patients with ALL without arthropathy vs ALL with arthropathy

Characteristics	Pre-B-ALL n = 102	Pre-B $ALL_{arthropathy}$ n = 26	*P* value
Female, No. (%) % (n/N)	42% (43/102)	65% (16/26)	.02
Standard risk, % (n/N)	51% (52/102)	30% (8/26)	.17
Intermediate risk, % (n/N)	27% (28/102)	35% (9/26)	.32
High risk, % (n/N)	22% (22/102)	23% (6/26)	.59
Blasts in peripheral blood, % (n/N)	75% (76/102)	50% (13/26)	.13
Age, y	4.3 (2.7-8.7)	5.6 (3.4-8.0)	.52
Hemoglobin, g/dL	7.1 (5.3-8.7)	9.5 (6.9-11.3)	.001
Platelets, $\times 10^9$/L	39 (14-74)	117 (31-175)	.001
WBC, $\times 10^9$/L	8.7 (3.8-39)	7.5 (2.7-18)	.35
Neutrophils, $\times 10^9$/L	0.3 (0.1-0.7)	0.7 (0.3-1.4)	.02
Monocytes, $\times 10^9$/L	0.3 (0.0-1.0)*	0.4 (0.1-2.6)	.46
Lymphocytes, $\times 10^9$/L	5.6 (2.6-15)*	4.3 (2.4-11)	.41
CRP, mg/L	20 (7-46)*	51 (15-114)	.02
ESR, mm/H	82 (48-109)*	65 (38-91)†	.44
S100A9, pg/mL	37 (21-77)	51 (20-122)	.06
S100A12, pg/mL	67 (20-191)	216 (123-528)	<.001
TNF-alpha, pg/mL	7.3 (4.4-14)	10 (5.4-18)	.42
IL-1 beta, pg/mL	1.3 (1.1-2.3)	1.5 (0.8-2.3)	.88
IL-4, pg/mL	13 (10-16)	12 (10-14)	.26
IL-6, pg/mL	3.0 (1.8-6.0)	3.8 (1.5-6.5)	.79
IL-10, pg/mL	4.8 (2.0-12)*	2.4 (0.9-5.5)†	.004
IL-12 p70, pg/mL	20 (10-26)	23 (13-26)	.46
IL-13, pg/mL	90 (79-99)	82 (74-97)	.40
IL-17, pg/mL	1.7 (1.1-2.0)*	1.1 (1.1-2.0)†	.19
IL-18, pg/mL	353 (225-570)	287 (184-423)	.13
MMP-3, pg/mL	1351 (955-2390)	1366 (1060-2736)	.63
MPO, pg/mL	335 (170-963)	986 (292-3072)	.01
CCL-2, pg/mL	350 (247-558)	449 (185-549)	.75
CD25, pg/mL	1211 (920-1626)	1069 (724-1698)	.34

Values are expressed as median with IQR in parentheses unless otherwise stated.
*Missing values, ALL: monocytes and lymphocytes n = 114; CRP: n = 112; ESR: n = 42; IL-10: n = 109; IL-17: n = 75.
†Missing values $ALL_{arthropathy}$: ESR: n = 18; IL-10: n = 22; IL-17: n = 17.

Table III. Comparison of the clinical characteristics with the level of the biomarkers in JIA (n = 236) vs ALL (n = 150) and JIA vs $ALL_{arthropathy}$ (n = 27)

Characteristics	Normal ranges	JIA n = 236	All n = 150	*P* value	$ALL_{arthropathy}$ n = 27	*P* value
Females, No. (%)		166 (70)	67 (45)	<.001	18 (67)	.826
Age, y		6.0 (2.9-10.3)	4.6 (2.9-9.3)	.493	5.7 (3.6-9.5)	.959
Hemoglobin, g/dL		11.7 (11.3-12.8)*	7.9 (5.6-10.0)	<.001	9.7 (7.7-11.1)	<.001
Platelets, $\times 10^9$/L		331 (287-415)*	48 (20-115)	<.001	144 (47-178)	<.001
CRP, mg/L		0 (0-10)*	21 (7-51)†	<.001	33 (20-97)	<.001
ESR, mm/H		12 (6-24)*	65 (39-104)†	<.001	68 (39-99)	<.001
S100A9, pg/mL	471 (15-1771)	511 (315-1281)	44 (11-106)	<.001	47 (30-113)	<.001
S100A12, pg/mL	4419 (256-10917)	925 (434-1610)	106 (33-356)	<.001	221 (124-528)	<.001
TNF-alpha, pg/mL	3.5 (2.4-30)	9.1 (1.7-17)	8.2 (4.4-17)	.312	9.2 (5.4-18.1)	.312
IL-1 beta, pg/mL	4.6 (3.5-22)	17 (7-30)	1.3 (1.1-2.3)	<.001	1.5 (0.8-2.3)	<.001
IL-4, pg/mL	10.4 (2.7-11.4)	89 (55-133)	12 (10-16)	<.001	12 (10-14)	<.001
IL-6, pg/mL	17 (0.53-42)	6.4 (3.2-13)	2.9 (1.7-5.5)	<.001	3.8 (1.5-6.5)	.030
IL-10, pg/mL	3 (0.23-20)	5.3 (2.3-7.1)	4.4 (1.8-9.8)†	.295	2.4 (1.1-5.7)‡	.130
IL-12 p70, pg/mL	*	13 (0.5-72)	20 (10-26)	.758	23 (13-26)	.940
IL-13, pg/mL	*	497 (387-621)	90 (79-97)	<.001	82 (79-97)	<.001
IL-17, pg/mL	3.6 (0.5-8.7)	9.7 (0.8-16)	1.6 (1.1-2.0)†	<.001	1.1 (1.1-2.0)‡	<.001
IL-18, pg/mL	23 (3.5-489)	206 (136-292)	330 (213-557)	<.001	278 (146-423)	.040
MMP-3, pg/mL	3351 (11.2-8645)	5174 (1747-16460)	1360 (977-2528)	<.001	1415 (1050-2528)	<.001
MPO pg/mL	*	2289 (1593-3561)	569 (218-1568)	<.001	975 (292-1957)	<.001
CCL2, pg/mL	3.8 (5-19)	180 (112-251)	349 (234-543)	<.001	449 (185-549)	<.001
CD25, pg/mL	2324 (7.3-21918)	590 (421-766)*	1117 (824-1587)	<.001	1021 (724-1587)	<.001

Values are stated as median with IQR unless otherwise stated. *P* values are calculated by Mann–Whitney *U* test, except for categorical variables calculated by Fisher exact test (*P**).
*Missing values, JIA: hemoglobin: n = 201; platelets: n = 203; CRP: n = 198, ESR: n = 196, CD25: n = 202.
†Missing values, ALL: CRP: n = 139; ESR: n = 58; IL-10: n = 131; IL-17: n = 92.
‡Missing values, $ALL_{arthropath}$: ESR: n = 18; IL-10: n = 22; IL-17: n = 17.

Table IV. Comparison of clinical characteristics and level of biomarkers between pre–B-ALL (n = 128) vs nonsystemic JIA (n = 225) and systemic JIA (n = 11)

Characteristics	Pre–B-ALL n = 128	Nonsystemic JIA n = 225	*P* value	Systemic JIA n = 11	*P* value
Female, No. (%)	59 (46)	158 (70)	<.001	7 (64)	.21
Age, y	4.3 (2.7-8.6)	6.5 (2.9-10.4)	<.001	4.7 (1.5-11.0)	.65
Hemoglobin, g/dL	7.4 (5.3-9.5)	11.9 (11.3-12.9)†	<.001	11.3 (9.7-11-4)	<.001
Platelets, $\times 10^9$/L	43 (16-101)	330 (287-408)†	<.001	367 (206-756)	<.001
CRP, mg/L	9 (4-41)	0 (0-0)†	<.001	25 (10-62)	.98
ESR, mm/H	0.4 (0.2-1.0)	12 (6-22)†	<.001	28 (9-80)	.02
S100A9, pg/mL	40 (21-85)	508 (314-1087)	<.001	1403 (362-5381)	<.001
S100A12, pg/mL	78 (28-250)	904 (433-1605)	<.001	1845 (526-8256)	<.001
TNF-alpha, pg/mL	7.6 (4.6-14.2)	9.1 (2.3-17)	.60	2.0 (1-27)	.24
IL-1 beta, pg/mL	1.3 (1.1-2.3)	17 (6.2-30)	<.001	17 (12-30)	<.001
IL-4, pg/mL	13 (10-16)	89 (57-133)	<.001	94 (5-185)	.11
IL-6, pg/mL	3.1 (1.8-6.3)	6.5 (3.2-13)	<.001	6.3 (2.1-39)	.08
IL-10, pg/mL	4.1 (1.8-9.8)	5.3 (2.3-7.1)	.37	6.4 (3.4-10.4)	.75
IL-12 p70, pg/mL	20 (10-26)	13 (0.5-72)	.70	28 (0.5-94)	.60
IL-13, pg/mL	90 (79-97)	497 (387-621)	<.001	546 (461-678)	<.001
IL-17, pg/mL	1.7 (1.1-2.0)	9.2 (0.8-16)	<.001	16 (0.8-35)	.005
IL-18, pg/mL	329 (218-557)	200 (136-276)	<.001	2046 (315-6721)	.005
MMP-3, pg/mL	1360 (970-2553)	5119 (1712-16389)	<.001	10 296 (2557-60797)	<.001
MPO pg/mL	451 (187-1197)	2286 (1534-3505)	<.001	2392 (1973-4659)	<.001
CCL-2, pg/mL	359 (246-554)	183 (112-255)	<.001	149 (60-184)	<.001
CD25*, pg/mL	1155 (873-1643)	588 (419-766)†	<.001	660 (441-883)	<.001

*Missing values ALL: CRP: n = 139; ESR: n = 58; IL-10: n = 131; IL-17: n = 92.
†Missing values JIA: hemoglobin: n = 192; platelets: n = 191; CRP: n = 186, ESR: n = 184, CD25: n = 191.

correlation between IL-1 beta, IL-4, IL-12p70, IL-13, IL-17, and MMP-3 (correlation coefficients 0.4-0.8, $P < .001$). Further, S100A9, S100A12, and platelets had a strong positive correlation with MPO (correlation coefficients 0.4-0.6, $P < .001$), Figure 1. The correlations did not differ for pre-B-cell ALL and T-cell ALL. In JIA, the correlations were less prominent, although the same trends occurred (Figure 2). We found no significant correlation between active or cumulative joints and the biomarkers in ALL or JIA. For the JADAS71 score, we found weak positive correlations between S100A12, IL-4, IL-12p70, and IL-13 (correlation coefficients 0.19-0.25). The correlations did not differ for systemic and nonsystemic JIA.

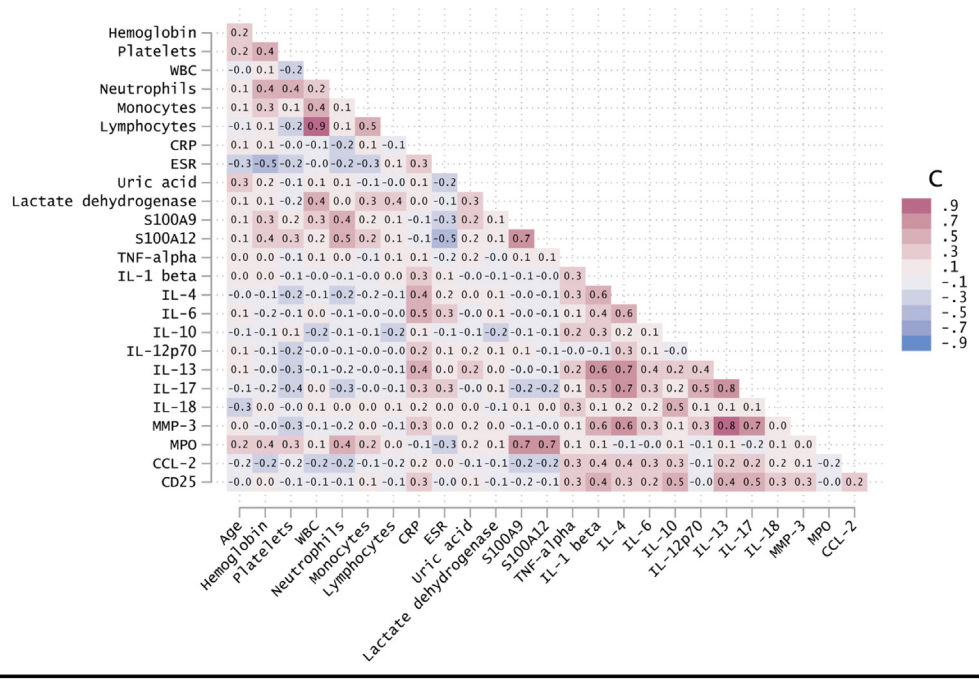

Figure 1. Heat plot illustrating the correlations between age, laboratory values, and the biomarkers evaluated by Spearman correlation coefficient (c) for the acute lymphoblastic leukemia cohort (n = 150).

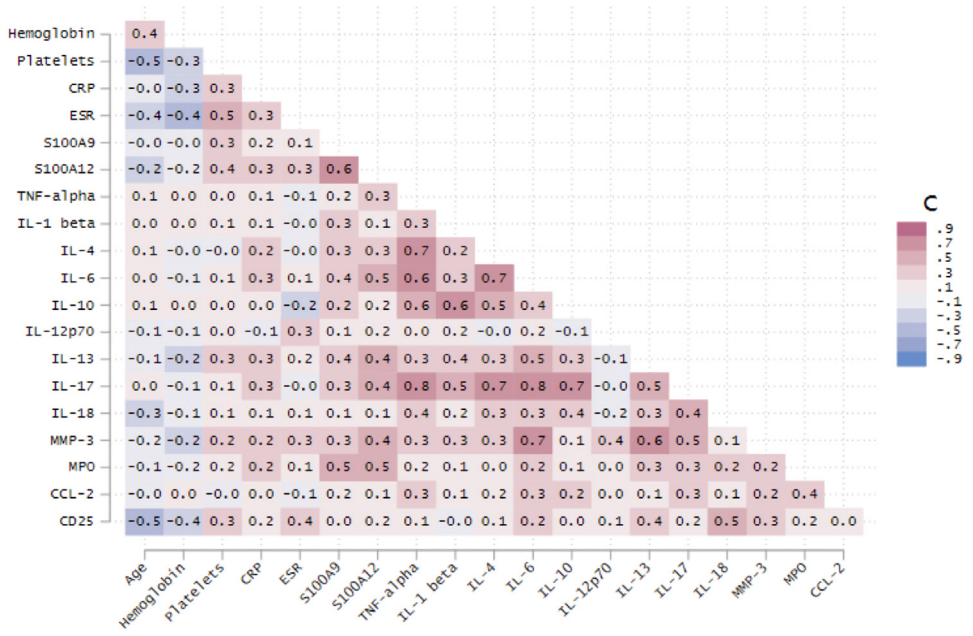

Figure 2. Heat plot illustrating the correlations between age, laboratory values, and the biomarkers evaluated by Spearman correlation coefficient (c) in the cohort with juvenile idiopathic arthritis (n = 236).

As the focus of this study is the child with ALL mistaken for nonsystemic JIA, and since the level of the biomarkers differed for both T-cell ALL vs pre-B-cell ALL and for systemic vs nonsystemic JIA (Table I), we excluded the 22 children with T-cell ALL (including just one with arthralgia) and the 11 children with systemic JIA from the predictive analyses. First, we calculated the cross-validated, recalibrated, and age-adjusted AUC values on the individual performance for each of the biomarkers. Then, for the best markers, we evaluated the predictive performance with optimal cut-off values illustrated with AUCs and predicted probabilities (Figure 3 and Table V). For IL-13, there was no overlap in the serum levels for ALL and JIA resulting in an AUC of 100% (95% CI 100%-100%). Further, IL-4 and S100A9 had a high predictive performance with AUCs of respectively 99% (95% CI 97%-100%) and 98% (95% CI 94%-99%), Figure 3. The predictive performance of IL-4, IL-13, and S100A9 exceeded the predictive performance of both hemoglobin, platelets, CRP, and ESR (Table V). Further, S100A12, IL-1beta, IL-12p70, and CD-25 revealed high AUCs of 86-90%, although

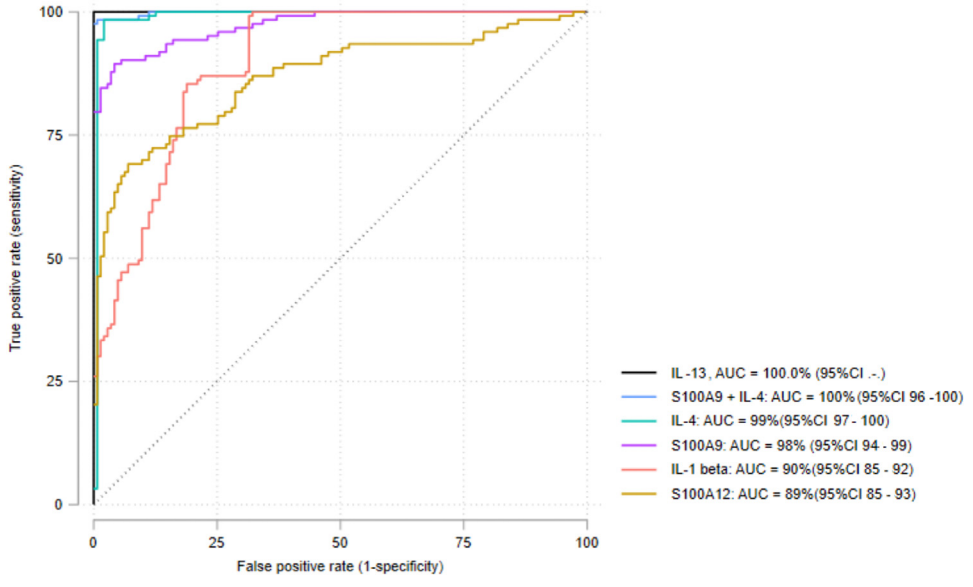

Figure 3. Receiver operating characteristics curves illustrating the AUC model for the prediction of ALL.

Table V. The predictive performance of the best biomarkers compared with the existing laboratory values (hemoglobin, platelets, CRP, and ESR) to detect pre–B-ALL (n = 128, hereof 26 with arthropathy) from nonsystemic JIA (n = 225)

Biomarkers	Optimal cut-off	AUC (95% CI)	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
IL-13	<140 pg/mL	100% (100%-100%)	100% (100%-100%)	100% (100%-100%)	100% (100%-100%)	100% (100%-100%)	100% (100%-100%)
IL-4	<23 pg/mL	99% (97%-100%)	97% (95%-99%)	99% (96%-100%)	95% (89%-98%)	97% (94%-99%)	98% (93%-100%)
S100A9	<230 pg/mL	98% (94%-99%)	93% (89%-96%)	95% (90%-97%)	91% (84%-95%)	93% (89%-97%)	92% (86%-96%)
Platelets	$<278 \times 10^9$/L	97% (92%-98%)	96% (93%-98%)	98% (95%-99%)	92% (86%-96%)	95% (91%-98%)	97% (92%-99%)
Hemoglobin	<12 g/dL	93% (88%-96%)	89% (85%-93%)	94% (90%-97%)	82% (74%-88%)	89% (83%-93%)	90% (84%-95%)
ESR	>15 mm/H	92% (83%-94%)	87% (82%-91%)	93% (88%-96%)	60% (46%-75%)	91% (86%-94%)	68% (51%-82%)
IL-1beta	<4 pg/mL	90% (85%-92%)	79% (74%-84%)	71% (64%-78%)	90% (83%-94%)	90% (84%-95%)	70% (62%-77%)
S100A12	<864 pg/mL	89% (85%-93%)	83% (78%-87%)	86% (81%-91%)	77% (68%-84%)	86% (81%-91%)	77% (68%-84%)
IL-12p70	>36 pg/mL	88% (83%-91%)	79% (74%-83%)	83% (78%-88%)	71% (62%-79%)	83% (78%-88%)	71% (62%-79%)
CRP	>1 mg/L	87% (79%-89%)	77% (72%-82%)	84% (78%-89%)	65% (55%-74%)	81% (75%-86%)	69% (59%-78%)
CD-25	>458 pg/mL	86% (80%-90%)	81% (76%-85%)	90% (84%-94%)	68% (59%-76%)	81% (75%-86%)	81% (72%-88%)

NPV, negative predictive value; *PPV*, positive predictive value.
Estimated from 10-fold cross validation, which has been recalibrated and computed using a threshold of 0.5 for the predicted probabilities of ALL.

with relatively low specificities and negative predictive values, which was also the case for ESR and CRP (**Figure 3** and **Table V**). Finally, when simply calculating the sensitivity and specificity of IL-13 being below 140 for the 26 children with pre-B-cell ALL vs nonsystemic JIA, such revealed a sensitivity of 100% (95% CI 87%-100%) and a specificity of 100% (95% CI 98%-100%).

Discussion

The value of inflammatory biomarkers as diagnostic tools to differentiate the child with ALL and arthropathy from the one with nonsystemic JIA has previously been evaluated to a very limited extent.[50] To advance understanding of the utility of inflammatory biomarkers, our study evaluated and identified S100A9, IL-4, and IL-13 as well-performing diagnostic markers, with AUC and predicted values exceeding both platelets, hemoglobin, CRP, and ESR. Recently, we evaluated the use of lectin complement pathway proteins as markers to differentiate ALL with arthropathy from JIA in the present cohort. We revealed M-ficolin as a valuable marker with an AUC of 94% (95% CI 83%-100%), exceeding CRP and hemoglobin.[50] In the present study, the AUCs for S100A9, IL-4, and IL-13 were 95%-100%, including high accuracies ranging from 93% to 100%, which indicates even higher diagnostic values than M-ficolin.

Identifying the child with ALL and arthropathy from JIA is challenging when hematological indices are close to normal and in the absence of blasts in peripheral blood.[2,3] Children with ALL and arthropathy present with few clinical and laboratory signs of leukemia compared with ALL without arthropathy,[1] and we confirmed higher levels of hemoglobin, platelets, and neutrophils in this subgroup. The levels of the biomarkers were equally low whether or not the child had arthropathy or blasts in peripheral blood, strengthening the diagnostic value.

The levels of the proinflammatory proteins S100A9 and S100A12 were higher in the subgroup with $ALL_{arthropathy}$ compared with ALL without arthropathy in the present study, which is in accordance with previous findings of S100A9 and S100A12 as important markers of the inflammatory process in arthritis.[14,15,22,56] These phagocyte-specific S100 proteins are expressed in the cytoplasm of granulocytes and early stages of monocytes and can operate as strong pro-inflammatory cytokine-like molecules as do endogenous ligands of Toll-like receptor 4, when released from stressed or damaged cells.[16,57,58] Several studies have found S100AA9 and S100A12 elevated in JIA compared with healthy controls,[17-20,23,24] which we confirmed. Furthermore, we revealed low levels of S100A9 and S100A12 in ALL being low compared with the normal ranges and markedly lower than in JIA.

IL-4 and IL-13 have multiple functions, including regulating Th2-mediated immunity, stimulating B-cell proliferation, and activating eosinophils, basophils, and mast cells.[40,59,60] IL-4 and IL-13 have been stated as important elements of the pathogenesis of rheumatoid arthritis,[39,40] and have been suggested to play a significant role in the downregulation of the inflammatory process and beneficially modulate the course of the disease.[41-44] Spadaro et al found serum levels of IL-13 to be significantly higher in patients with rheumatoid arthritis than in healthy individuals.[61] We found high levels of IL-4 and IL-13 in children with JIA compared with children with ALL.

Functionally, cytokines have been classified as either proinflammatory or anti-inflammatory.[29] However, the specific role of individual cytokines during inflammation or injury can shift from beneficial to harmful depending on the nature of the pathologic state in which a cytokine is involved and its target cell population.[29,62] Further, cytokine–cytokine interactions play a crucial role during immunological and inflammatory responses resulting in additive, antagonistic, or synergistic activities.[62,63] We found high correlations between many of the cytokines, and as the correlation coefficients were above 0.7 among 2 or more predictors, there might be an element of collinearity as illustrated in the heat plots, which complicates the possible use of these markers as individual predictors.

Systemic JIA is an aggressive autoinflammatory disorder with extensive activation of the innate immune system, likely influenced by an imbalance between proinflammatory cytokines and immune deactivators.[14,58,64] Presentation of systemic JIA may be bewildering with many differential diagnoses due to clinical characteristics involving fever, affected hematologic parameters, enlargement of the liver and spleen, and rash.[64] In the present study, we found significantly higher levels of S100A9, S100A12, and IL-18 in systemic vs nonsystemic JIA, which is in accordance with previous findings.[65,66] These three markers have been suggested as valuable predictors to distinguish the child with systemic JIA from groups of other diseases that can be easily misdiagnosed as systemic JIA, here among ALL.[14,22,25]

There are some limitations to the study design and the analytic setup, which may have influenced the protein levels and conceivably impaired the predictive abilities. The first issue is the element of missing data. The number of patients with unavailable serum samples entails a risk of selection bias in both cohorts and reduced generalizability. In addition, the fact that we did not have any data on WBC or neutrophils in the JIA cohort is an important limitation, as neutrophils especially have been identified as a valuable marker to differentiate ALL from JIA.[3,67] Second, the JIA serum samples were collected up to 6 months after disease onset, with some patients being on disease-modifying antirheumatic drugs and having an inactive disease. However, the results show that most inflammatory markers were still higher in JIA than in ALL (and presumably would even be higher in untreated, active JIA), thereby implying a risk of underestimating the difference between the groups. Third, analyzing cohorts of patients with rare diseases is associated with some limitations regarding the interpretation of data due to the relatively low number of individual patients in subgroup analysis. Only 7 children had arthritis in the group of children with ALL, and only 11 had systemic JIA. Fourth, the level of the biomarkers might have been influenced by both a long storage period and the dilution of the serum samples for the JIA cohort. These issues may have influenced the protein levels and conceivably impaired their predictive abilities.[68,69] Finally, the predictive performance of S100A9, IL-4, and IL-13 should be repeated in another nonselected cohort, including external validation, to ensure that the diagnostic performance is reproducible. External validation in future studies would ensure that the diagnostic performance of S100A9, IL-4, and IL-13 is reproducible, ultimately evaluated in clinical trials before final conclusions can be drawn and before implementations in clinical practice would be recommended.

In conclusion, our results indicate that S100A9, IL-4, and IL-13 may be used as serum markers to distinguish pre-B-cell ALL from nonsystemic JIA. However, our prediction models require validation, repeating the study in another nonselected cohort. ■

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