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Research Article

Secretory Phospholipase A2, Type IIA, A Biomarker of Septic Arthritis In Children With Joint Irritability

Irma T. Ugalde*, Halim Hennes, Lawson Copley, Rong Huang, M. Douglas Baker

Pediatric Emergency Medicine, Baylor College of Medicine, USA
Pediatric Emergency Medicine, UTSouthwestern School of Medicine, USA
Department of Orthopedics, UTSouthwestern School of Medicine, USA
Deparment of Statistics, Childrens Medical Center, USA
Pediatric Emergency Medicine, John Hopkins Children's Medical Center, USA

***Corresponding author**
Ugalde, Pediatrics Section of Emergency Medicine, Department of Pediatrics, Baylor College of Medicine, 6621 Fannin St. A.2210, Houston, TX 77030, Tel: 832-824-6525; Fax: 832-825-5424; Email: Ugalde76@gmail.com

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Abstract

Background: Discerning between early septic arthritis (SA) and transient synovitis (TS) is a challenge. Objective: Examine the relationship between secretory phospholipase A2 type IIa (SpLA2) and SA in children presenting with acute non-traumatic limp or joint pain. Methods: A prospective pilot study was conducted on a convenience sample of children presenting with acute non-traumatic limp or joint pain to a tertiary care emergency department. Clinical and laboratory data including SpLA2 levels were collected. Children were compared based on final diagnosis of SA vs. TS and the sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were determined. Results: In the 149 enrolled children, 85 were diagnosed with SA (true or presumed) or TS. The difference in median serum concentration of SpLA2 in children with SA (136,141) and TS (2,884) was statistically significant ($p<0.001$.) The difference in median serum concentration of SpLA2 in children with true SA (190269) and TS (2,884) was statistically significant ($p<0.001$.) Receiver Operator Characteristic (ROC) curve analysis revealed that SpLA2 was more accurate in predicting SA than the total white blood cell (WBC), Sedimentation rate (ESR), and C-reactive protein. An SpLA2 value of 22,658 pg/dL had a sensitivity of 88%, specificity of 88%, positive predictive value of 65%, negative predictive value of 97% and positive and negative likelihood ratios of 7.5 and 0.13, respectively. Conclusions: SpLA2 may play a role in early diagnosis of children with SA. Verification is warranted in a larger cohort.

INTRODUCTION

Young children commonly present with limp or joint pain to the emergency department (ED). While the condition is often a benign process such as toxic synovitis (TS), a small percentage may have septic arthritis (SA), which must be diagnosed and managed promptly to avoid irreversible joint damage and disability [1-4]. The clinical presentations and standard laboratory markers in children with early SA and TS are similar, making it difficult to distinguish between the two. Currently there is no single test that can accurately discriminate SA from TS. Furthermore, there is currently no universally agreed upon clinical and laboratory criteria to ascertain a diagnosis, short of joint aspiration for synovial fluid analysis.

Secretory phospholipase A2 Type IIa (spLA2) is an enzyme made and secreted by a number of cells including synovial fluid cells in response to inflammatory stimuli [5]. High levels of spLA2 are found in adults and children with bacterial infections [6-8] In addition, murine models have demonstrated that articular damage occurs from the host's immune response, and that severity of arthritis correlates with cytokines such as IL-6, IL1B, and IL18 in the joint [9]. Downstream from these cytokines is Secretory Phospholipase A2 Type IIA (SpLA2), which is believed to mediate more distal effects of inflammation. In one study, serum sPLA2 levels were correlated with disease activity in Juvenile Rheumatoid Arthritis [10]. Furthermore, intra-articular injection of SpLA2 in rats causes acute synovitis [5]. Furthermore the more rapid onset of action of this inflammatory marker

compared to CRP, make it more attractive in diagnosing early septic arthritis.[7] We postulate that measuring SpLA2 in children with limp or hip irribility may help to differentiate those patients with early SA versus TS. The purpose of this study is to evaluate the ability of SpLA2 to discriminate between the two disease entities.

METHODS

This is a prospective convenience sampling of children 0-18 years old seen in a tertiary care ED from April 2, 2010 to April 10, 2011, presenting with single joint pain or swelling in which septic arthritis was suspected in the ED setting. Exclusion criteria included immunodeficiency, rheumatologic or oncologic disease, post-operative infections, clinical sepsis, traumatic musculoskeletal injury with suspected fracture and subjects without a guardian present to sign the informed consent in English or Spanish. Children over the age of 12 years provided written assent. The study protocol was approved by the institutional review board. Data collection included demographics, fever presence, and joint involved. Standard testing included: blood culture, complete blood count with differential (CBC), C-reactive protein (CRP), and sedimentation rate (ESR). One additional mL of serum was obtained for SpLA2 levels (Cayman Chemical Laboratory;Ann Arbor, Michigan); results were not available to the treating physician. Children were classified as having SA or TS based on Kocher's published definitions with slight modification. In our study, true SA was defined as a patient with synovial fluid or peripheral blood cultures positive for known pathogens regardless of synovial fluid cell count, presumed SA was defined as a patient with synovial fluid white blood cell count, s-WBC > 50,000/mm^3, and negative cultures, and TS was defined as a patient having s-WBC < 50,000/mm^3, negative cultures, and resolution of symptoms without antibiotics or surgical intervention. Kocher's definition of true SA differed from ours in that it also included s-WBC > 50,000/mm^3 in addition to positive blood or synovial fluid cell culture [11]. Otherwise, definitions were the same for the other two classes of outcomes, presumed SA and TS. Each participant received telephone follow-up at 1 month and 6 months post-enrollment and records were reviewed for subsequent healthcare visits after the ED stay.

SAS 9.2 (SAS Institute, Inc., Cary, NC.) was used for analyses. Descriptive analyses were performed using proportions, frequency distributions, means, and confidence intervals. Receiver operating characteristic (ROC) curves were created to identify appropriate threshold for a diagnostic test. Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV), and positive and negative likelihood ratios were calculated to evaluate the diagnostic test.

RESULTS

One hundred fifty children presented with single joint pain or swelling: 1 declined, 64 received diagnoses other than SA or TS, and 85 were included: 68 TS, 10 confirmed SA, and 7 probable SA. Furthermore, of the true SA, 5 were hips, 4 knees, and 1 ankle. In the presumed SA group, 5 were hips, 1 elbow, and 1 knee. In the transient synovitis group, 59 were hips and 9 knees. The 10 children with confirmed SA grew *S. aureus* (5), *S. pyogenes* (2), and 1 each grew *Brucella melitensis*, *Bordetella helmisii*, and H. *influenzae*. The demographic, clinical, and laboratory findings of the study population are described in Table 1a and 1b. There were elevations in SpLA2, ESR, and CRP (but not WBC) in those with presumed and confirmed SA compared to those with TS. None of the patients in the TS group were ultimately diagnosed with a rheumatologic or infectious process on follow-up phone interviews or chart review through 6 months post initial ED presentation. One patient in the PSA group was ultimately believed to have a rheumatologic process despite treatment with long term antibiotics. All patients were from the Dallas, Texas area and none had reported travel to Lyme endemic areas.

We evaluated two previous risk scores for septic arthritis in our cohort (see Table 2). This table shows the number of previously published risk factors for SA present in each of our patients. The first section analyzes the number of risk factors present in our subjects according to the Kocher analysis of fever > 38.6 C, non-weight bearing status, ESR> 40 mm/h, and serum WCC > 12 x 10^9, while the next section adds CRP > 2mg/dL as a fifth predictor based on Caird's analysis [11,13]

Extrapolating from the ROC curve supports that the test performs well above the value of 63,117 pg/dL and below the value of 22,658 pg/dL (see figure 1a and 1b.) A value < 22,658 pg/dL essentially rules out SA. Conversely, a value > 63,117 pg/dL would be concerning for SA.

The sensitivity, specificity, positive and negative predictive values, and likelihood ratios were calculated for SpLA2 at various cut off points, table 3.

DISCUSSION

Clinicians have used a variety of clinical, laboratory, and radiographic data for distinguishing between children with SA and TS. These scores primarily were contingent on availability of joint aspiration and occurred in populations where pre-

Table 1a: Characteristics of TS vs. SA+ Presumed SA patients.

	TS	SA + Presumed SA	p value
Sample Size	68	17	
Median Age (years) [a]	4	8	0.037
Male [b]	63.2%	52.9%	0.58
Race [b]			1
White/Non-Hispanic	14.71%	11.76%	
White/Hispanic	63.24%	70.59%	
Black/Non-Hispanic	11.76%	12%	
Other Non-Hispanic	2.94%	0%	
Other Hispanic	7.35%	6%	
Median Duration of Symptoms (days) [a]	1	2	0.10
Median Fever days [a]	0	1	<0.0001
Fever (temperature > 38.6) [b]	4.41%	58.82%	<0.0001

[a]: p value is from the Wilcoxon Rank Sum Test
[b]: p value is from the Fisher's Exact Test
CRP: C-reactive protein; d: days; ESR: erythrocyte sedimentation rate; SA: septic arthritis; s-WBC: synovial fluid white blood cell count; TS: toxic synovitis; WBC: white blood cell count; y: years

Table 1b: Characteristics of Children with TS vs. true SA patients.

Variable		Total Population (n=78)	True SA (n=10)	TS (n=68)	p-value
Demographics	% male		50%	63.2%	0.42
	Median age (y)		7.5	4	0.18
	Race:				1
	White/Non-Hispanic		10.0%	14.7%	
	White/Hispanic		80.0%	63.2%	
	Black/Non-Hispanic		10.0%	11.8%	
	Other Non-Hispanic		0%	2.9%	
	Other Hispanic		0%	7.4%	
Symptoms	Median symptom duration (d)		4	1	0.005
	Median fever days (d)		2	0	<0.0001
	Febrile %		80.0%	4.4%	<0.0001
Laboratory Evaluation* (median and interquartile range)	WBC (cells/mm^3)		10.5 (7.0 – 14.0)	9.4 (8.0 – 11.0)	0.83
	ESR (mm/hr)		37.5 (27.0 -75.0)	9.0 (7.0 – 17.5)	<0.0001
	CRP (mg/dL)		14.7 (8.7 -18.8)	0.10 (0.10 – 0.75)	<0.0001
	SpLA2 (pg/dL)		190269 (63117 - 245258)	2884 (1870 – 8791)	<0.0001

CRP: C-reactive protein; d: days; ESR: erythrocyte sedimentation rate; SA: septic arthritis; s-WBC: synovial fluid white blood cell count; TS: toxic synovitis; WBC: white blood cell count; y: years

Table 2: Comparing our cohort to historical risk factors for SA.

		# of factors	All SA (n=17) # (%)	Confirmed SA (n=10) # (%)	Probable SA (n=7) # (%)	TS (n=68) # (%)
Predicted probability of SA	Kocher criteria[3]	0	3 (18%)	2 (20%)	1 (14%)	10 (15%)
		1	3 (18%)	0	3 (43%)	42 (62%)
		2	3 (18%)	2 (20%)	1 (14%)	16 (23%)
		3	7 (41%)	5 (50%)	2 (29%)	0
		4	1 (6%)	1 (10%)	0	0
	Caird criteria[5]	0	1 (6%)	1 (10%)	0	10 (15%)
		1	2 (12%)	1 (10%)	1 (14%)	40 (59%)
		2	3 (18%)	0	3 (43%)	15 (22%)
		3	4 (24%)	3 (30%)	1 (14%)	3 (4%)
		4	6 (35%)	4 (40%)	2 (28%)	0
		5	1 (6%)	1 (10%)	0	0

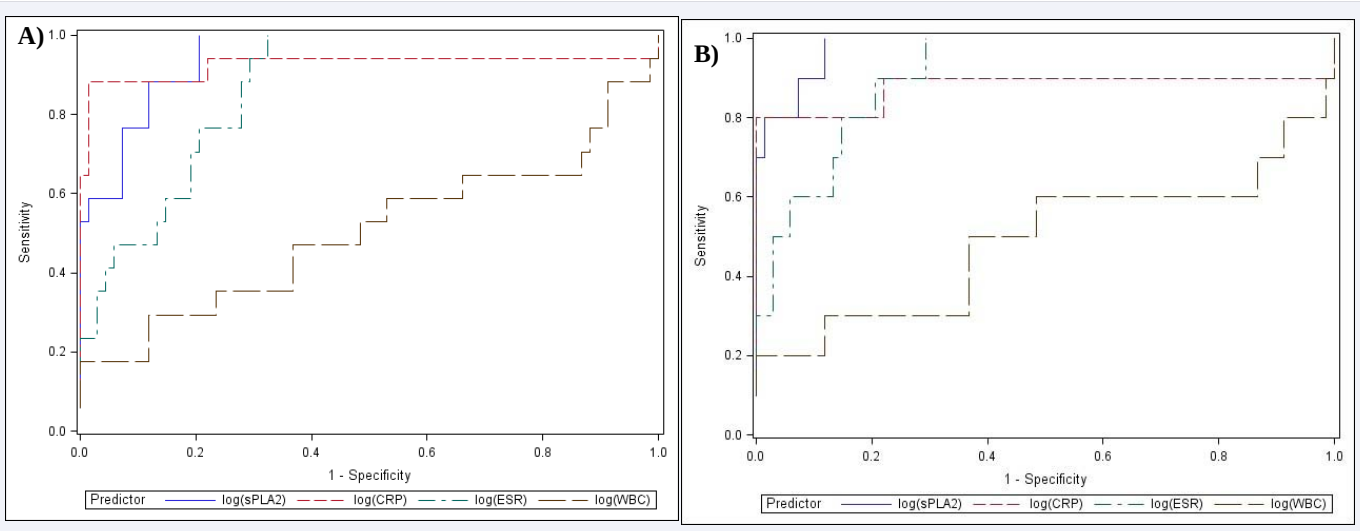

Figure 1 **A)** All septic arthritis (true and presumed) vs. toxic synovitis. Receiver operator characteristics (ROC) curves for sPLa2, CRP, ESR, and WBC. **B)** True septic arthritis vs. toxic synovitis. Receiver operator characteristics (ROC) curves for sPLa2, CRP, ESR, and WBC.

Table 3: Diagnostic power of SpLA2 at different cut off points.

sPLA2 (pg/dL)	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Positive Likelihood ratios	Negative Likelihood Ratios
63117	0.58824	0.9853	0.90909	0.90541	40.01633	0.4179
31994	0.76471	0.9265	0.72222	0.9403	10.40422	0.25396
24830	0.82353	0.8823	0.63636	0.95238	6.99686	0.20001
22658	0.88235	0.8823	0.65217	0.96774	7.4966	0.13334
9612	1	0.7941	0.54839	1	4.85673	0

test probability of SA was high. However, the sensitivities and negative predictive values have been suboptimal [11,12].The addition of CRP increases sensitivity, but has been studied in fewer children [13].

We demonstrated that among children presenting with limp, SpLA2 concentrations differentiate SA and TS. These results support our hypothesis that an increased serum concentration of sPLA2 is associated with septic arthritis and may be a useful screening tool.

While attempts to externally validate Kocher's criteria have not met with the accuracy determined in the original study, Kocher and Caird's utility of the CRP together, is the most useful clinical tool to date to help differentiate SA from TS [11,12,13]. However, when we analyzed our patients in terms of number of these historical predictors present (history of fever, ESR > 40 mm/hr, WBC > 12.0 x 10^9 cells/L, and non-weight bearing), nearly one fifth of patients with SA (true + presumed) would have been categorized as very low risk with 0/4 predictors from the Kocher study. Moreover, two patients in the true SA group that would have been categorized as very low risk with 0/4 predictors and essentially missed by the Kocher criteria would have been diagnosed appropriately using SpLA2. These children had levels of 35,673 pg/dL and 136,141 pg/dL, respectively, so if using the cut off value of 22,658 pg/dL as noted above, both cases would have been suspected. In the presumed SA group, one child with 0/4 predictors had a level of 31,994 pg/dL, which would have at least led to a joint aspiration. In fact, a total of 6 of the 17 patients in the SA group (true + presumed) had less than or equal to 1/4 predictors present, putting them at very low risk for SA. All but one of these patients would have been suspected of having SA with sPLA2 alone (see below for discussion of one outlier, patient with level of 9,612 pg/dL). Furthermore, when greater than 3/4 predictors were present, the risk was only 47% for SA. When a fifth predictor was added, the percentage of patients at risk for SA was only 41% with greater than 4/5 present risk factors.

The main limitation to this study is the small sample size. Consequently, we are unable to control for potential confounding variables such as anti-inflammatory use, antibiotic exposure, or varying physician practices (ie. threshold for obtaining laboratory markers on patients presenting with limp or joint pain may vary per clinical practice). A larger sample size could allow detection of a difference between the AUC for CRP, ESR, and sPLA2. Nevertheless, the study has the advantage of testing a clinical predictor in a general population (not all children had enough clinical suspicion to indicate a joint aspiration), rather than a carefully selected group referred to a tertiary care center as in prior studies. The study was conducted in an area not endemic for Lyme disease; it is unclear if it could be generalized to areas with spirochete etiologies for arthritis.

Furthermore, the standard definition of SA, true or presumed, is not without flaws. The reported synovial fluid culture rates in patients with true SA is about 30% [14,15,16]. Blood cultures are positive in about 20% of cases[15]. In turn, the standard definition of presumed septic arthritis based on a synovial fluid WBC count of > 50 x 10^3 may under or overestimate the number of cases in this group. In fact, gross analysis of the synovial fluid may be more accurate than cell count in establishing a diagnosis of SA [17]. In one retrospective study, those with synovial fluid WCC of >50 x 10^3 and >100 x 10^3 had a diagnosis of SA in 47% and 77% of patients, respectively. Patients with a synovial WCC of < 50 x 10^3 have a reduced likelihood of infection [18]. In the current study, this is evident in two of the ten cases of SA, Haemophilus influenzae and Brucella melitensis with synovial WBC of 22,658 and 35,673, respectively. Thus our definition of true septic arthritis veered from that of Kocher to include a positive pathogen in the blood or synovial fluid despite unmet criteria of the synovial fluid cell count which is an insensitive marker [19]. In turn, the definition of the presumed septic arthritis group which is taken from the Kocher criteria in our study leaves us in a bit of a conundrum but it is the current standard and all these patients were treated clinically for SA with prolonged antibiotics.

Of note, one patient within the presumed SA group based on a synovial fluid WCC of >50 x 10^3, presented over a year later with a similar complaint of joint pain and was found to have an elevated synovial fluid WCC and again treated presumptively for SA. A rheumatology consultation during the second hospitalization diagnosed both episodes with TS. When the patient was enrolled in the study during the first presentation, she was found to have an ESR and CRP that were 24 mm/h and 3.3 mg/dl, respectively, and 2/5 risk factors from the Caird study, placing the patient at high risk for SA. The sPLA2 at <10 x10^3 pg/dl, however, was most discerning if the rheumatology expert opinion is the correct one.

Another limitation of this study is that we did not limit our study to only hip complaints but included all single joint presentations with suspicion for possible septic arthritis. This may confound the findings although it is still a viable clinical dilemma to try to rule out a septic joint in an inflamed joint regardless of location. The majority of the joints were hips. While a convenience sample may not be optimal for inclusion of all possible cases in a year, we felt this was reasonable for a pilot study.

CONCLUSIONS

Serum concentrations for SpLA2 are higher in patients with

SA than in those with TS. It is a more accurate predictor of SA than the WBC count. SpLA2 may be a useful tool, either alone or in combination with other markers, in identifying children presenting with limp or joint pain.

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