

Determination the Cut off Level for Synovial S-Tream1 to Differentiate Septic from Aseptic Arthritis: A Cross Sectional Study, Tehran, Iran

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Abstract

Objective: To evaluate the usefulness of STREM-1 level in synovial fluid (SF) to differentiate septic from aseptic arthritis.

Methods: A cross sectional study performed in the Pediatrics ward of Rasoul Hospital, Tehran, IRAN (2008-2009). Out of 66 children, 53 synovial fluid (SF) samples studied. Direct gram stain, conventional and Bactec culture, quantification of STREM-1 level (EIA Quantikine, R&D systems, USA) had done. STREM-1 levels compared between septic (n=26) and aseptic (n=27) arthritis. Chi square values (CI 95%, p<0.05) were considered statistically significant.

Results: Septic arthritis diagnosed in 26 cases; *S. Aureus* (7/18, 38%). SF-STREM-1 Cut off level 825 pg./ml yielded 50% sensitivity, 70% specificity, 64% PPV, 64% NPV. The AUC was 0.603 (95% CI; 0.448-0.757, $P = 0.2$). SF-STREM-1 Levels were higher in patients with bacterial arthritis in compare with aseptic arthritis (95% Confidence Interval Odds Ratio 9.852-1.039; fisher exact test; P value: 0.056).

Conclusion: SF-STREM-1 level even in very low amount (825 pg/ml) had intermediate (50%) sensitivity for diagnosis of septic arthritis. 70% specificity is excellent and sufficient for definite diagnosis, but it could misdiagnosed just in 30% of septic arthritis cases (from other inflammatory arthritis), 64% NPV or the test is a limited factor. In our opinion the presence of STREM-1 in SFA can potentially assist clinicians in the diagnosis of half but not all cases with bacterial arthritis. The Positive SF culture as gold-standard test for diagnosis would obtain up to 80%. Combination of new biologic markers (PCT and sTREM-1) in SFA could be more helpful in high suspicious cases with negative culture (already on antibiotic treatment; or normally under growth of SF culture).

Keywords: STREM-1 (Soluble triggering expressed on myeloid cells-1); Synovial fluid (SF); Arthritis

Introduction

Bacterial arthritis is one of the most potentially serious infectious occurring to infants and older children with a high rate of acute complications and risk of long-term morbidity [1,2].

Laboratory investigations play an important role in the diagnosis and follow-up of inflammatory rheumatic diseases in children [3].

An accurate and rapid diagnosis of bacterial arthritis is essential for earlier treatment and a good outcome. Signs and symptoms are often nonspecific and it is not always possible to make a differential diagnosis between bacterial and aseptic arthritis [3,4]. The gold-standard test for diagnosis is Sculptured which are positive in 80 % of cases. Gram staining of SF reveals bacteria in about 50 to 80 % of cases but is an insensitive technique and must be confirmed by culture. SFA leukocyte count and concentration of protein and glucose lack specificity and sensitivity for the diagnosis [1,2]. The sensitivity of PMN predominance for aseptic arthritis was 57% whereas the specificity was 10%. The positive predictive value for PMN predominance in aseptic disease is 81% but the negative predictive value is 31% [4]. PCR based molecular techniques provided the increase of the diagnosed bacterial etiologies for clinical specimens with negative bacterial culture [5].

Recently various biological markers like CRP, procalcitonin, or STREM-1 was useful for diagnosis and to differentiate between

bacterial and non-bacterial infection. A soluble form of TREM-1 (STREM-1) is released from the activated phagocytes and can be found in body fluids. Previous studies have shown that STREM level rises in some infectious diseases. High level of STREM-1 detected in the blood of patients with endotoxemia and sepsis, meningitis or other rheumatic diseases. Authors considered it to differentiate infectious from other inflammatory causes [6-10]. Nonetheless, recent studies have shown STREM-1 rises even in other inflammatory processes [11-13]. Bacterial Arthritis continues to be a most important illness with high morbidity among unvaccinated (*S. pneumonia* and *H. influenza* type b) patients in Iran [15,16]. Definite and rapid diagnosis of septic arthritis is needed in our children.

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The aim of this study was to evaluate the usefulness of STREM-1 level in synovial fluid (SF) to differentiate between septic and aseptic arthritis.

Methods and Materials

A cross sectional study performed in the Department of Pediatrics, Rasoul Hospital, Tehran, Iran between 2008 and 2009. The study was approved by the Ethical Committee in Research Center of Pediatric Infectious Diseases in Tehran University of Medical Sciences. Out of children with fever and mono arthritis, 53 cases ranged between 5 months-16 years; mean age: 11 ± 3.8 years. 53.4% male, 46.6% female studied.

Definition of bacterial arthritis

Arthritis + positive SF culture or positive blood cultures +/- SF culture; positive Gram stains.

Confirmatory diagnostic tests (PCR) were not available in cases with strong clinical suspicion of infection or other conditions with SF inflammatory processes (reactive arthritis).

Exclusion criteria

We excluded all cases with poly arthritis; migratory poly arthritis; Rheumatic fever; immune deficiencies state; Brucellosis, hemarthrosis, TB arthritis.

Data collection

After informed consent, a questionnaire was completed by an authorized physician for each case (e.g. age, gender, analysis of SF samples, biochemical parameters, gram stain, SF culture in both convention and Bactec medium), SF studied for bacterial culture, direct gram stain; 0.5-3 ml of SFA stored at -70°C until assayed. STREM-1 level (pg/ml) in SFA measured by quantitative sandwich enzyme immunoassay technique (Human TREM-1 immunoassay, Quantikine, R&D systems, USA).

The investigator performing the assay was unaware of the SFA culture results.

Statistical analysis

The student's *t* test was used to determine significant in means for continuous variables. The mann-whitney test and the chi-square test were used to compare groups. P-values less than 0.05 were considered statistically significant.

A receiver-operating – characteristic curve (ROC) constructed to illustrate various cut-offs of STREM-1 levels in differentiating between groups. Statistical calculation was performed with SPSS statistical software (version 15.5; SPSS Inc.). McNemar test and computing kappa statistics used for comparing the diagnostic value SF-Stream level with culture results. Kappa statistics was interpreted as $\kappa > 0.75$ excellent agreement, $\kappa < 0.4$ poor agreement and κ between 0.4 and 0.75 fair to good agreement.

Results

Septic arthritis diagnosed in 26 cases (18 positive culture; 8 positive gram stain).

The most common organisms included *S. aureus*: 7/18 (38%) was the most common cause; *S. pneumoniae*:5, *H. influenzae*:2, *Klebsiella*:1; *N. meningitis*:1; *P. aurogenosa*:1; *C. albicans*:1.

Amount of SF -STREM-1 Levels (n=53) presented in Figure 1.

The area under the ROC curve for discriminating between septic and aseptic arthritis was 0.603 (95% CI; 0.448-0.757, $P = 0.2$) (Figure 1).

Cut off level 825 pg./ml for SF-STREM-1 yielded 50% sensitivity, 70% specificity, 64% Positive Predictive Value (PPV), 64%, Negative Predictive Value (NPV). Poor agreement observed between SF -STREM-1 levels and positive SF culture (P value: 0.05; Kappa=0.2) (Table 1).

The test result variable(s): STREM has at least one tie between the positive actual state group and the negative actual state group.

At the smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values (Table 2).

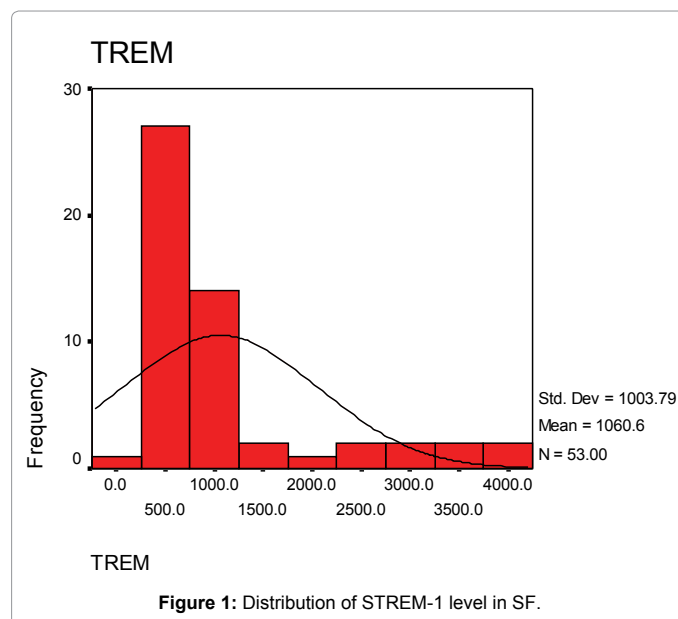
SF -STREM-1 Levels (800pg/ml) compared between septic (26) and aseptic arthritis (27) cases (Table 3).

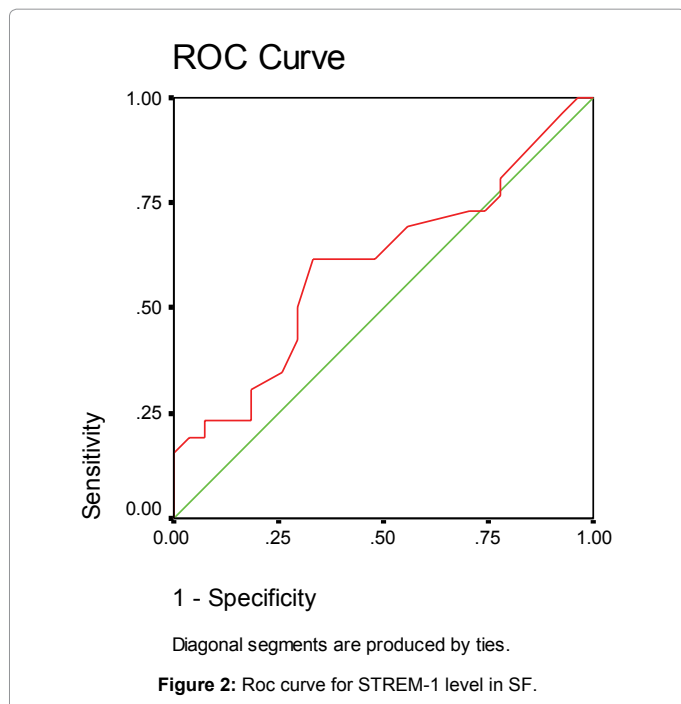
SF -STREM-1 Levels were higher in patients with bacterial arthritis in compare with aseptic arthritis (95% Confidence Interval Odds Ratio 9.852-1.039; fisher exact test; P value: 0.056)

Discussion

Bacterial arthritis diagnosed in 27% of cases with acute onset arthritis. We observed relatively low number of laboratory confirmed cases of bacterial arthritis in cases with strong clinical suspicion of infection or cases with inflammatory arthritis. Unfortunately the confirmatory diagnostic tests were not available for ruling out those cases with negative cultures.

Previous antibiotic treatment might explain false negative cultures in some cases but most of cases with negative culture; Negative gram stain categorized as inflammatory non-septic or reactive arthritis. Reactive arthritis is more frequent than bacterial arthritis in childhood [1-3]. As we expected the incidence of septic arthritis is far from previous Iranian studies (45%) in adult cases [13]. But its incidence is very lower than 70% in pediatric population in our country. The older age of the cases (mean age: 11 ± 3.8 years) studied in present study or undefined inclusion criteria for selection of cases could explain this





Variable	SF-stream-level (pg./dl)
Cut off level	825 pg./ml
Sensitivity (%)	50%
Specificity (%)	70%
Positive predictive value (%)	64%
Negative predictive value (%)	64%
Kappa Index	0.28
Area under the ROC Curve	0.603 (0.448-0.757)

Table 1: Statistical variables for SF /STREM-1 level.

1 – Specificity	Sensitivity	Positive if Greater Than or Equal To(a)
1.000	1.000	199.0000
.963	1.000	225.0000
.926	.962	275.0000
.778	.808	350.0000
.778	.769	425.0000
.741	.731	455.0000
.704	.731	480.0000
.556	.692	550.0000
.481	.615	625.0000
.444	.615	675.0000
.333	.615	750.0000
.296	.500	825.0000
.296	.423	875.0000
.259	.346	950.0000
.185	.308	1100.0000

Table 2: Test Result Variable(s): TREM.

Total	SEPTIC		SF -STREM-1 Levels (>800pg/ml)
	Negative	Positive	
25	9	16	Positive
28	18	10	Negative
53	27	26	Total

Table 3: Comparison the SF -STREM-1 Levels (>800 pg/ml) between septic and aseptic arthritis".

differences [14]. Many authors reported 80% positive SF cultures in at best methods [2-4] Laboratory investigations play an important role in the diagnosis and follow-up of inflammatory rheumatic diseases in children. The sensitivity of PMN predominance for aseptic arthritis was 57% whereas the specificity was 10%. The positive predictive value for PMN predominance in aseptic disease is 81% but the negative predictive value is 31% [2].

S. aureus as the most common cause is similar to all previous Iranian studies [8,13,14] and also Wang et al. (Taiwan) and Li et al. [4] studies in other countries Wang et al. [1] reported *S. aureus* as the predominant causative organism in 43% (n=58) septic arthritis (mean age =3 years) which is very close to ours.

Breda et al. [3] showed routine laboratory tests are useful to confirm a suspected diagnosis, to assess disease activity, and to measure the response and toxicity to treatment.

Li et al. [4] concluded that laboratory tests do not rule out septic arthritis with accuracy in 73 adult cases with septic arthritis. They reported sensitivities of an elevated WBC (>11,000 cells/mm), ESR (>30 mm/hr), or SFA-WBC (> 50,000 cells/mm), 48%, 96%, and 64%, respectively.

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a membrane molecule that is expressed on the surface of neutrophils and monocytes. S TREM-1 mediates the acute inflammatory response to microbial products [5-10].

The use of biological markers, especially PCT, lymphokines and acute-phase proteins, has been proposed to facilitate the accuracy of the initial diagnosis and in discriminating bacterial arthritis from aseptic arthritis [15-18].

Some authors reported that soluble adhesion molecules are increased in sepsis, SIRS and meningitis [6-10].

Determann et al. [6] explained the CSF- S TREM-1 level (cutoff level 20 pg./ml) had 73% sensitivity, 77% specificity (AUROC curve 0.82) for diagnosis of meningitis in adults. 78% sensitivity and excellent (100%) specificity of test in meningitis cases reported by Bishara et al. [7].

Barati et al. [8] reported that C-reactive protein and ESR had better results than serum STREM-1 and white blood cell count, in distinguishing between septic and non-infectious SIRS sepsis. Gonzales-Roldan et al. [9] reported STREM-1 is regulated post-transcriptionally and its ligand in the sera of some septic patients. The probable mechanism of SF-STREM-1in arthritis is similar to meningitis. Intra synovial shedding in addition to diffusion through blood-SF barrier caused the high level of STREM-1 in SF [9-11]. Gibot et al. [19] determined the usefulness of procalcitonin (PCT) and STREM-1 determinations in the diagnosis of nosocomial sepsis.

Here, we observed the higher amount of SF -STREM-1 Levels in patients with bacterial arthritis, Poor agreement obtained between SF -STREM-1 levels and positive SF culture (P value: 0.056; Kappa=0.2). SF-STREM-1 level (>800 pg./ml) yielded 50% sensitivity, 70% specificity, 64% Positive Predictive Value (PPV), 64%, Negative Predictive Value (NPV). All above studies upon STREM-1 level in meningitis had better results than septic arthritis.

The SF-STREM-1 level had better results than 50% reported for both culture and gram staining in patients who are already on antibiotic treatment [1]. False negative culture in studied septic arthritis cases could explain poor agreement observed between SF-STREM-1 levels

and positive culture (P value: 0.037; Kappa=0.28). 70% specificity of SF STREM-1 levels are excellent for differentiation of septic arthritis from other causes of arthritis if this complementary diagnostic test added to conventional SF culture. SF STREM-1 levels could not differentiate bacterial arthritis from other inflammatory process in 30% cases. It could be a better test in high suspicious cases that are already on antibiotic treatment (negative culture).

It postulated that an elevated STREM-1 level may be a useful clinical marker to direct the clinician to the diagnosis of septic arthritis. However, except to Collins et al study; STREM-1 levels varied from one diagnostic category to another [11].

Collins et al. [11] like as ours, identified that STREM-1 was elevated both in rheumatoid arthritis and septic arthritis but not in gouty arthritis, non-septic/non-RA inflammatory arthritis and non-inflammatory arthritis. Bohnsack et al. [12] found elevated serum levels of soluble CD154 in children with juvenile idiopathic arthritis Shiari et al. [13] compared the sTREM-1 level in serum and synovial fluid of children with juvenile rheumatoid arthritis and normal population. The average of sTREM-1 level in synovial fluid of patients was 160.5 pg/ml which was lower than 825 pg./ml in present study. Like here, they found no relation between elevated sTREM-1 levels and other acute phase reactants (ESR, CRP and WBC of serum- and- Platelet and WBC of synovial fluid). They conclude that elevated sTREM-1 serum and synovial fluid level is not only attributable to infection but also to other inflammatory processes as like as JIA. The rise of sTREM-1 in serum and synovial fluid were parallel to each other [13].

In setting of an acute arthritis in present study, 50% sensitivity and 70% specificity for diagnosis of bacterial arthritis is not so far from serum PCT (>0.5 mg/mL) which had 55% sensitivity and 94% specificity. Also; CRP (>50 mg/L) had 100% sensitivity; but poor specificity (40%) [15-17].

Martinot et al. [16] reported synovial PCT was not useful to discriminate between infectious and noninfectious arthritis in clinical practice but serum PCT was the best parameter to distinguish patients with acute bacterial arthritis from patients with crystal induced arthritis or rheumatoid arthritis. They achieved 55% sensitivity; 94% specificity for serum PCT (>0.5 ng/mL) [16]. In Butbul-Aviel et al. [17] study, PCT was found to be a useful marker in the diagnosis of osteomyelitis but not in septic arthritis. Sensitivity 53.3% for serum PCT test was very close to present study. Serum PCT appeared to be higher in patients with septic arthritis resulting from "systemic infection" than in cases resulting from direct inoculation [17]. Fottner et al. [18] compare the serum procalcitonin to differentiate septic and nonseptic arthritis.

Although specific markers of bacterial arthritis might be a good parameter to distinguish patients with acute bacterial arthritis from rheumatoid arthritis but in regard to increasing the sensitivity of test for definite diagnosis of septic arthritis, we offer adding the PCT to STREM-1 level in SF. In our opinion, use of synovial STREM-1 level with 50% sensitivity in association with other acute phase responses (PCT; ESR; CRP etc.) could nevertheless be useful in an emergency situation for the diagnosis of bacterial arthritis [15-18]. Combination of both tests would help more in rapid diagnosis especially in high suspicious cases who are already on antibiotic treatment (negative culture). Prevention of unnecessary prolonged antibiotic therapy in cases with inflammatory arthritis will achieved.

Strength of the Study

This data is critical as all further calculations regarding sensitivity,

specificity, etc. are all based on the assumption that these patients have been correctly categorized to culture or gram stain. These results would be valuable information as there does not appear to be a similar study looking at the levels of STREM-1 in children with arthritis. Answer to a complex question of the utility of SF/ STREM-1 level in children with arthritis in this investigation rather than simply describing (as Collins did) obtained.

Limitations of the Study

Small population study especially in younger age (<2 years) is an important limitation. This is further complicated by the relatively low number of laboratory confirmed cases of bacterial arthritis in the study. The undefined Serum sTREM-1 level in cases is the other limitation. Some studies explained the parallel rising of sTREM-1 in serum and synovial fluid.

Conclusions

SF-STREM-1 level even in very low amount (825 pg./ml) had intermediate (50%) sensitivity for diagnosis of septic arthritis. 70% specificity is excellent and sufficient for definite diagnosis, but it could misdiagnosed just 30% of septic arthritis cases (from other inflammatory arthritis). Indeed, 64% Negative Predictive Value for the test is a limited factor.

In our opinion the presence of STREM-1 in SFA can potentially assist clinicians in the diagnosis of half but not all cases with bacterial arthritis. The Positive SF culture as gold-standard test for diagnosis would obtain up to 80%. Confirmatory diagnostic tests (PCR) test would be helpful for decreasing the false negative cultures with strong clinical suspicion of infection or other inflammatory conditions.

Combination of new biologic markers (PCT and sTREM-1) in SFA could be more helpful in high suspicious cases with negative culture (outpatient cases were already on antibiotic treatment; or normally under growth of SF culture). A larger group of patients needed to be studied to confirm our findings.

Acknowledgments

This study was supported by the Research Center of Pediatric Infectious Diseases, Tehran University of Medical Sciences. This study was approved by the Ethical Committee in the research center of pediatric infectious diseases in Tehran University of medical sciences.

Ethical Considerations

Ethical Committee in the Research Center of Pediatric Infectious Diseases (affiliates by Tehran University of Medical Sciences) has reviewed and approved the Waiver of Authorization for use of protected health information (PHI) for research purposes for the following study.

Principal Investigator: Dr Samileh Noorbakhsh, MD associate professor, Pediatric Infectious Disease; Research Center of Pediatric Infectious Diseases, Tehran University of Medical Sciences.

Title: Searching the STREM-1 (Soluble triggering expressed on myeloid cells-1) level in synovial fluid of arthritis.

Date of Approval: May 2008.

The following PHI for which use or access is requested has been determined to be necessary for the conduct of the study [Insert the patient information to be used or disclosed, or attach documentation of the information].

1. The use or disclosure of PHI involves no more than minimal risk.
 - Granting of waiver will not adversely affect privacy rights and welfare of the individuals whose records will be used.
 - The project could not practicably be conducted without a waiver.
 - The project could not practicably be conducted without use of PHI.

- The privacy risks are reasonable relative to the anticipated benefits of research.
- An adequate plan to protect identifiers from improper use and disclosure is included in the research proposal.
- An adequate plan to destroy the identifiers at the earliest opportunity, or justification for retaining identifiers, is included in the research proposal.
- The project plan includes written assurances that PHI will not be re-used or disclosed for other purposes.
- Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

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