

## Identification of bacterial antigens and super antigens in synovial fluid of patients with arthritis: a cross sectional study

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### Abstract

**Background:** An accurate and prompt diagnosis of bacterial arthritis is essential for earlier treatment and a good outcome. Superantigens produced by Staph. Aureus are among the most lethal toxins. The paper objective was Identification of common bacterial antigens and S.aureus superantigens in synovial fluid (SF) of children with negative culture and direct smear for other bacteria except for S.aureus.

**Methods:** In this cross-sectional study a total of 62 patients with a mean age of  $11 \pm 3.8$  years (range: 5 months-16 years) with acute arthritis in pediatric and orthopedic wards of Rasoul Hospital (2008-2010) were studied. Three common bacterial antigens (e.g. S.pneumonia, H.influenza, N. meningitis) using LPA (latex particle antigen) and Staphylococcal superantigens (TSST1; Enterotoxin A; B; C) using ELISA method (ABcam; USA) were identified in 60 adequate SF samples with negative culture and negative direct smears (for other bacteria except for S.aureus. Staphylococcal superantigens were compared with S.aureus infection (positive culture or direct smear).

**Results:** Positive bacterial antigens (LPA test) were found in 4 cases including two S. Pneumonia, one N.meningitis, and one H.influenza. S.aureus was diagnosed in 7 cases including 4 positive cultures and 3 positive smears. Staphylococcal superantigens (toxins) were found in 73% of SF samples. Some cases had 2 or 3 types of toxins. S.aureus toxins were reported in 47% of culture negative SF samples. Positive TSST1, Enterotoxin B, Enterotoxin A, and Enterotoxin C were found in 47% (n= 28), 18% (n= 10), 39% (n= 22), and 39% (n=21) of cases respectively. The most common type of superantigens was TSST1; and Enterotoxin A was the less common type. Except for Enterotoxin A, no relation between positive S.aureus culture and positive tests for superantigens in SF was found.

**Conclusion:** S.aureus has a prominent role in septic arthritis. S.aureus toxins might have a prominent role in arthritis with negative SF culture. Rapid identification of bacterial antigens (LPA) or S.aureus superantigens (toxins) are valuable for diagnosis in cases with negative cultures. We recommend usage of complementary methods (e.g. antigen detection tests) in children. Those tests are cheaper and easier in comparison with PCR as a complex and time-taking method. Identification of S.aureus superantigens in SF of all cases with negative culture, or treatment with antagonist drugs needs further clinical trial studies.

**Keywords:** Septic arthritis, Arthritis, Bacterial antigens, Superantigens

### Introduction

Bacterial arthritis is one of the most potentially serious infectious diseases occurring in infants and older children with a high rate of acute complications and risk of long-term morbidity (1-3). An accurate and

prompt 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 (4-5). SF's (Synovial fluid) leukocyte count and concentra-

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tion of protein and glucose lack specificity and sensitivity for the diagnosis of meningitis. Lee et al evaluated 73 adults with septic arthritis confirmed by positive arthrocentesis culture or operative findings and reported that elevated WBC (>11,000 cells/mm), ESR (>30 mm/hr.), or SF-WBC (> 50,000 cells/mm) had a sensitivity of 48%, 96%, and 64%, respectively. Laboratory tests do not rule out septic arthritis with accuracy (6, 7).

Gram staining of SF reveals bacteria in about 50 to 80 % of cases but it is an insensitive technique and must be confirmed by culture. Latex agglutination tests were adapted for rapid and direct detection of soluble bacterial antigens (H.influenza type b, S. Pneumonia, group B streptococci, and N. meningitis) in SF. Latex particle agglutination tests (LPA) is limited to patients with SF pleocytosis who have received prior antimicrobial therapy (1, 2, 6). The gold-standard test for diagnosis is SF culture which is positive in 80 % of cases (3). PCR (Polymerase Chain Reaction) based molecular techniques has been led to an increase in diagnosis of bacterial etiologies for clinical specimens with negative bacterial culture (7, 8). Rosary et al replaced Real-time PCR as a sensitive and reliable technique (7, 8). Real-time PCR is easier to interpret and allows detection of more cases than conventional PCR but it is very expensive and unavailable (7, 8).

Staphylococcal superantigens have been implicated in the pathogenesis of inflammatory diseases. Superantigens produced by S.aureus are among the most lethal of toxins which trigger an excessive cellular immune response leading to toxic shock (9-11). Chemokines have an important role in arthritis. Chemokines are small, chemo attractant cytokines which play key roles in the accumulation of inflammatory cells at the site of inflammation (10) Schutyser et al reported that monocytic cells respond to Gram-positive bacterial infection by the production of CCL18/PARC (Chemokine Ligand 18/Pulmonary and Activation-Regulated Chemokine) in the synovial

cavity (12). Recently, the antagonist activity of this peptide has been identified as the novel domain in superantigens that is critical for their toxic action (13-15).

Bacterial arthritis continues to be the most important illness with high morbidity among unvaccinated (S. pneumonia and H. influenza type b) Iranian children (16-18). Recently various biological markers like CRP, procalcitonin, or STREM-1 (Soluble Triggering Receptor Expressed on Myeloid cells) reported to be utilized for differentiation of bacterial and aseptic arthritis in Iran (17,18). Definite and prompt diagnosis of bacterial arthritis is essential in our patients (19-20). In this study, SF samples examined for gram staining, bacterial culture, and biochemical testing; LPA test was done for three common pathogens (S. Pneumonia, H.influenza, N.meningitis) and Staphylococcal superantigens (TSST1, Entrotoxin A;B;C).

### Methods

In this cross-sectional study a total of 60 patients with acute arthritis admitted in pediatric and orthopedic wards of Rasoul hospital (2008-2010) in Tehran were evaluated. This study was approved by the Ethics Committee of Tehran University of Medical Sciences.

A questionnaire was completed by a single physician for 67 consecutive children with arthritis admitted. (Variables including: age, gender, analysis of SF samples, biochemical parameters, direct smear by gram stain, LPA and SF culture in both convention and Bactec medium /or universal bacterial PCR).

Excluded cases: 7 cases with positive culture, direct smear, or PCR (for bacteria except for S. aureus) were excluded, including S.Pneumonia (3 cases), N. meningitis (1 case), H. influenza (1 case), P. aureogenosa (1 case), and K. pneumonia (1 case).

Included cases: 60 cases with negative culture and direct smear in adequate SF samples were selected. The SF examined for three common bacterial antigens (S. pneumonia, H. influenza, N. meningitis) by

LPA (Latex Particle Antigen) method and staphylococcal superantigens (Enterotoxin A, B, C, and TSST1) using ELISA method (ABcam; USA).

Statistics analysis: Data analysis were conducted using SPSS (version 11.5). The Students' T test was used to determine significant differences in means for all continuous variables. Chi-square values (CI 95%,  $p < 0.05$ ) were calculated for all categorical variables.  $p < 0.05$  was considered significant. McNemar and computing kappa statistics used for comparing between variables.

### Results

Mean age of the cases with arthritis was  $11.66 \pm 3.8$  years with a range of 5 months to 16 years. 53.4% of cases were male and 46.6% were female. Two cases with arthritis had not adequate SF specimen.

Out of 67 SF samples obtained from arthritis cases, 60 samples with negative culture and negative direct smear tested for

bacterial antigens by LPA method. Positive LPA detected in 4 cases including *S. pneumonia* (2 cases), *N. meningitis* (1 case), and *H. influenza* (1 case). *S. aureus* diagnosed in 7 cases including four positive cultures and three positive smears.

All types of Staphylococcal superantigens (toxins) were positive in 73% of cases; some cases had 2 or 3 type of toxins. TSST1 was the most common and Enterotoxin A was the less common type of staphylococcal superantigens in SF samples. Positive TSST1, Enterotoxin B, Enterotoxin A, and Enterotoxin C in 47% ( $n=28$ ), 18% ( $n=10$ ), 39% ( $n=22$ ), and 39% ( $n=21$ ) of SF samples respectively.

Except for Enterotoxin A ( $p$  value=0.06, Kappa=0.6), no significant relation was observed between *S. aureus* infection and other positive staphylococcal superantigens in SF: TSST1 ( $p$  value=0.2; Kappa=0.37), Enterotoxin B ( $p$  value=0.1; Kappa=-0.15), Enterotoxin C ( $p$  value=0.5; Kappa=0.35) (Table 1).

Table 1. Correlation between positive *S. aureus* infection and staphylococcal superantigens in SF

Total	TSST1			p value=0.2 Kappa=0.37
	Negative	Positive		
4	1	3	Positive	<i>S. aureus</i> infection
9	6	3	Negative	
13	7		Total	
Total	Enterotoxin A			p value=0.06 Kappa=0.6
	Negative	Positive		
4	0	4	Positive	<i>S. aureus</i> infection
8	6	2	Negative	
12	6	6	Total	
Total	Entrotoxin B			p value=0.1 Kappa=- 0.15
	Negative	Positive		
4	4	0	Positive	<i>S. aureus</i> infection
8	7	1	Negative	
12	11	1	total	
Total	Enterotoxin C			p value=0.5 Kappa=0.35
	Negative	positive		
4	2	2	Positive	<i>S. aureus</i> infection
6	5	1	Negative	
10	7	3	Total	

## Discussion

In this study, positive SF culture was found in 34.5% of cases that was lower than 70% reported in pediatric population (1-3, 17). Positive SF culture has been reported up to 80 % (1, 2). The lower rate of microorganisms isolated in this study might be due to natural lack of growth of organism in synovial fluid, previous antibiotic usage, or technical methods. Similar to other local studies, *S.aureus* was the most common organism diagnosed in this study (18-19).

The isolated organisms are similar to Wang et al study (3). Predominant causative organism in 58 septic arthritis (mean age=3years.) studied in Taiwan was *Staphylococcus aureus* (43%), followed by coagulase-negative *Staphylococcus*, *S. pneumoniae*, *Salmonella* spp. *H.influenza* type b, and group B *Streptococcus* (3).

Identification of bacterial antigens (LPA) or *S. aureus* superantigens (toxins) is valuable for diagnosis in cases with negative SF cultures. Adding the latex agglutination test for detection of bacterial antigens, is also helpful for rapid and accurate diagnosis of bacterial arthritis. 2 The antigenic tests are cheaper and easier in comparison with PCR as a complex and time-taking method.

A possible role for staphylococcal toxins (superantigens) in arthritis was defined in present study. Staphylococcal superantigens (toxins) was found in 73% of SF samples; some cases had 2 or 3 types of toxins. *S.aureus* toxins reported in 47% of culture negative SF samples. Most common type of superantigens was TSST1; Enterotoxin A was the less common type. Except for Enterotoxin A, no significant relation was found between positive *S.aureus* culture and positive tests for superantigens in SF.

Superantigens produced by *S.aureus* are among the most lethal toxins which trigger an excessive cellular immune response leading to toxic shock (9-11). This study elucidates the pathogenic role for staphylococcal toxins (superantigens) in producing arthritis. Probably superantigens act with-

out direct invasion of the *S. aureus* organism in S.F (with negative culture).

Similar to this study, Floret et. al also discussed clinical aspects of streptococcal and staphylococcal toxic diseases. 10 Treatment of critical superantigens toxic effects with antagonist peptides is a novel domain in superantigens (12-15). Schutyser et al reported that monocytic cells respond to Gram-positive bacterial infection by the production of CCL18/PARC in the synovial cavity (12). Kaempfer et al defined the antagonist activity of this peptide against superantigens that is critical for its toxic action (13).

## Conclusion

*S.aureus* has a prominent role in septic arthritis. *S.aureus* toxins are detectable in SF of 47% of cases with arthritis. *S.aureus* toxins might have a prominent role in arthritis of cases with negative SF culture. Rapid identification of bacterial antigens (LPA) or *S.aureus* superantigens (toxins) are valuable for diagnosis in cases with negative cultures. We recommend usage of complementary methods (e.g. antigen detection tests) in children. Those tests are cheaper and easier in comparison with PCR as a complex and time-taking method.

Identification of *S.aureus* superantigens in SF of all cases with negative culture, or treatment with antagonist drugs needs larger clinical trial studies in future.

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