Assessment of synovial fluid and serum cytokine levels in children with septic arthritis

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Accepted 5 February 2021
Published Online First 12 February 2021

To cite: Dehority W, Plaster S, Helmig KC, *et al*. *J Investig Med* 2021;**69**:1059–1062.

ABSTRACT

Acute septic arthritis (ASA) is a common orthopedic infection of children which may produce devastating sequelae and chronic morbidity. Improved understanding of the intra-articular inflammatory response in ASA may identify cytokine targets with diagnostic or therapeutic potential, though no detailed investigations to this end have been performed. Given this, we used a multiplex cytokine assay for assessment of levels of 40 different cytokines in the synovial fluid and blood of children with ASA. Twelve children (8 controls undergoing orthopedic surgery for non-infectious conditions and 4 with ASA) were prospectively enrolled. Blood and synovial fluid were collected intraoperatively from each subject, and the levels of 40 cytokines were determined using a multiplex assay. Cytokines were organized by function and structure into 12 groups for analysis. The Benjamini-Hochberg method was used to control for type 1 errors, with an a priori false discovery rate of 10%. Subjects with ASA were younger than controls (mean age 8.0 vs 13.1 years, p=0.0400). Significant elevations were seen in interleukins (IL) with chemokine properties, IL-6 and those in the common-γ chain group in the blood and synovial fluid of children with ASA compared with controls, while significant elevations in 5 additional cytokine groups were seen in synovial fluid from children with ASA compared with controls, most notably IL-6 (median 8294.3 vs 10.7 pg/mL, p=0.0066). Our pilot study is the first to describe in detail the cytokine response in children with ASA, and highlights the need for additional study.

INTRODUCTION

Septic arthritis, one of the most common orthopedic infections in children, is a surgical emergency which may be associated with long-term complications and sequelae.[1] A robust intra-articular inflammatory reaction forms the basis for these sequelae, which results in part from a poorly defined cytokine response.[2] After intra-articular infection is established, an influx of inflammatory cytokines into the joint space leads to the release of matrix metalloproteinases and other enzymes which degrade collagen and may produce permanent joint damage.[1 2]

Our knowledge of the host intra-articular inflammatory response derives primarily from small studies of rheumatologic arthritides, with only a handful of reports studying septic arthritis in children (which have only assessed a very small number of cytokines with single-plex assays). However, interleukin (IL)-1β, IL-6 and tumor necrosis factor alpha (TNF-α) are known to be present in the synovial fluid of adults with non-gonococcal septic arthritis,[3] elevated levels of TNF-α and IL-1β are reported in the synovial fluid of children with septic arthritis[4] and IL-6 elevation may be present in *Kingella kingae* septic arthritis.[5]

Corticosteroids reduce mortality and disease severity in animal models of septic arthritis, with protection against cartilage destruction associated with an attenuated cytokine response.[6] Two randomized, placebo-controlled studies of dexamethasone in children with septic arthritis demonstrated significant improvements in clinical outcomes,[7 8] including reductions in residual joint dysfunction at 12 months.[7] However, steroids are a blunt instrument with broad immunological suppression. Hence, the idea of a selective blockade of potentially harmful intra-articular cytokine responses is appealing, though not studied. An improved understanding of cytokine expression in septic arthritis could identify cytokines associated with joint damage, help differentiate septic arthritis from non-infectious arthritides and monitor disease progression. However, to our knowledge, no study has characterized the cytokine response in either synovial fluid or blood in children with septic arthritis in detail. To this end, we performed a pilot study assessing the levels of 40 different cytokines in both the blood and synovial fluid concurrently in children with and without septic arthritis. We hypothesized we would see a unique cytokine profile in the synovial fluid compared with the blood of children with septic arthritis.

MATERIALS AND METHODS

Children ≤18 years of age were prospectively enrolled between March 7, 2018 and May 25, 2019. Immunocompetent subjects requiring orthopedic surgery for non-infectious conditions (eg, genu valgum repair) were enrolled as controls while immunocompetent children undergoing arthrotomy for septic arthritis were enrolled as cases. Blood and synovial fluid were obtained from both groups intraoperatively,

then centrifuged at 3500 rpm for 15 minutes and frozen at −80°C.

All specimens underwent cytokine analysis with the Bio-Rad 40-Plex cytokine assay on the Bio-Rad Bio-Plex 200 suspension array system (Bio-Rad Laboratories, Hercules, California). This system uses a bead-based Luminex technology permitting multiple protein analyses in a single sample, with results reported as median fluorescence intensity and concentration (pg/mL), along with simultaneous reporting of standards, controls, blanks, and cytokines in duplicate. The use of this system with synovial fluid is investigational.

Given that synovial fluid is exceptionally viscous and does not mix well with other fluids (Bio-Rad, personal communication), samples were passed through a 0.22 μL nylon membrane, the sample incubation time was increased to 1 hour and samples were treated with hyaluronidase (Sigma, 20 μ/mL) for 30 minutes at 37°C (to cleave glycosidic bonds in chondroitin sulfate), followed by centrifugation.[9]

Given the large number of cytokines assayed (40 total) and the complex interplay between them, we organized each into groups united by inherent features (eg, biochemical structure, common receptor usage and consistent physiological properties). Cytokines were grouped into (1) eotaxins (eotaxin 1, eotaxin 2, eotaxin 3)—involved in eosinophil recruitment[10]; (2) granulocyte colony-stimulating factor (GCSF) and granulocyte-macrophage colony-stimulating factor—responsible for granulocyte and antigen-presenting cell recruitment[11]; (3) the interferon-γ group (IL-28, IL-29, interferon-γ—with the latter assessed)—genetically similar and using the same receptor complex[12]; chemokines, responsible for leucocyte recruitment into inflamed tissues,[13] divided into (4) the CXC group (CXCL10, CXCL6, CXCL1, CXCL2, CXCL9, CXCL16, CXCL12)[14] and (5) the CC group (CCL2, CCL3, CCL25, CCL-17, CCL-23, CCL-19, CCL-20, CCL-15, CCL-22, CCL-13, CCL-7, CCL-1, CCL-27, CCL-21), with the CXC and CC groups differentiated by unique amino acid motifs near the N-terminus[14]; (6) macrophage migration inhibitory factor—inhibits apoptosis and mediates inflammation[15]; (7) the IL-1 group (IL-1β, IL-1ra)—produced from the same phagocytic cells and sharing a common three-dimensional structure[16]; (8) IL-10—maintains tissue homeostasis during infection by downregulating inflammatory responses, promoting tissue repair[17]; (9) the common-γ chain group (IL-2, IL-4, IL-7, IL-9, IL-15)—bind to the γc receptor; growth and proliferation factors driving lineage-specific differentiation[12]; (10) ILs with chemokine activity (IL-8, IL-16)—recruitment of inflammatory cells to sites of infection[12]; (11) TNF-α—regulates the magnitude of inflammatory responses[12]; and (12) IL-6—increases expression of hepatic acute-phase proteins and production of immunoglobulins.[12]

Means were calculated for each subject for every cytokine group (eg, the mean of the 7 cytokines in the CXC group), and the resulting mean values from each control subject were compared with the mean values for each case subject for each of the 12 cytokine groups for both blood and synovial fluid. Levels of individual cytokines were also compared between cases and controls for both serum and synovial fluid samples. A Mann-Whitney test was used for tests of statistical significance between cytokine values and a Welch's unpaired t-test was used for comparison of gender and age. Given the large number of comparisons undertaken, the Benjamini-Hochberg method was used to control for type 1 errors,[18] with a false discovery rate of 10% set a priori.

RESULTS

Twelve subjects were enrolled (8 controls, 4 cases). The blood from 1 case hemolyzed and was not analyzed. Cases were all male (p=0.0398), were younger than controls (mean age 8.0 vs 13.1 years, p=0.0400) and presented with a mean of 6 days (median 4 days) of symptoms prior to culture collection. All cases presented with progressively worsening joint pain (2 involving the hip and 2 the knee) and restricted range of motion of the affected joint. Two of the cases were unable to bear weight at the time of admission while the other 2 were limping. Fever was present in 2 cases (with maximum temperatures of 102°C and 103°C each). The other 2 cases did not have fever but were using ibuprofen regularly prior to admission for pain control. Per institutional practice, 1 mL of synovial fluid was collected for assessment of cell counts (1 case did not have synovial fluid cell counts assessed). The 3 cases with synovial fluid cell counts available demonstrated white cell values of 86.32×10^9 WBC/L (97% polymorphonuclear cells), 29.16×10^9 WBC/L (98% polymorphonuclear cells) and 136.70×10^9 WBC/L (93% polymorphonuclear cells). No nucleated red cells were noted in the synovial fluid from any case. All cases demonstrated resolution of symptoms following arthrotomy and antimicrobial therapy. The Kocher score for 3 cases was 3, and 2 for the other (with methicillin-susceptible *Staphylococcus aureus* isolated from a blood culture from the latter). A pathogen was not isolated from the other 3 subjects with septic arthritis. Median duration of antibiotic therapy was 47 days (IQR 39.5–55.0 days). One case suffered from concurrent osteomyelitis. At the end-of-therapy visit (median 57 days, range 31–151 days), all cases were well.

The only groups of cytokines with significant elevation in the serum of cases compared with controls were the ILs with chemokine properties (median 622.3 vs 119.6 pg/mL, p=0.0242), the common-γ side chain group (median 165.0 vs 67.6 pg/mL, p=0.0121) and the IL-6 group (median 526.8 vs 14.4 pg/mL, p=0.0186) (table 1). However, in synovial fluid, 7 cytokine groups demonstrated significant elevations compared with controls (table 2). This was most evident in the ILs with chemokine properties (median 1720.7 vs 73.5 pg/mL, p=0.0040), the common-γ side chain group (median 101.2 vs 17.7 pg/mL, p=0.0040) and IL-6 (median 8294.3 vs 10.7 pg/mL, p=0.0040) (table 2). The eotaxin, GCSF, IL-1 and TNF-α groups also demonstrated significant elevations compared with controls (table 2). Though the levels of many individual cytokines appeared significantly higher in cases versus controls, particularly in the synovial fluid (eg, IL-6, p=0.004 and eotaxin-1, p=0.0081), none of these differences retained statistical significance after correction for multiple comparisons.

DISCUSSION

We believe our pilot study is the first to conduct a detailed assessment of the cytokine response in synovial fluid and blood in children with septic arthritis. Previous work has

Table 1 Serum cytokine analysis in children with and without septic arthritis

	Control (pg/mL)			Case (pg/mL)			
Cytokine group	Mean	Median	Range	Mean	Median	Range	P value
CXC	661.5	678.9	540.4–785.9	1558.1	801.7	698.6–3174.1	0.0849
CCL	892.1	922.6	435.1–1202.3	1341.7	826.8	826.0–2372.3	1.000
Eotaxins	80.4	70.1	21.3–166.0	91.2	78.9	45.4–149.2	0.7758
MIF	180	114.2	36.0–411.3	936.7	967.8	165.8–1676.5	0.0849
GCSF	29.6	27.2	24.9–42.4	91.1	24.8	24.3–224.3	0.497
IL-1	348.5	328.4	66.0–722.1	245	240.9	102.9–391.2	0.6303
IL-10	26.9	28.2	16.5–34.8	109.9	27.3	25.0–277.5	0.6817
Interferon-γ	29.4	30.2	22.5–38.3	81.3	32	30.5–181.3	0.2196
Common-γ side chain	69.4	67.6	45.1–95.6	1358.5	165	146.2–3764.4	0.0121*
IL with chemokine properties	177.9	119.6	71.9–581.4	2572.2	622.3	258.3–6836.1	0.0242*
IL-6	15.9	14.4	10.3–24.0	7679.4	526.8	59.5–22 451.8	0.0141*
TNF-α	53.6	41.9	29.5–104.3	117.8	39.8	20.0–293.5	1.000

*Statistically significant; Mann-Whitney analysis used, with significance determined with the Benjamini-Hochberg correction (false discovery rate of 10%).
GCSF, granulocyte colony-stimulating factor; IL, interleukin; MIF, macrophage migration inhibitory factor; TNF, tumor necrosis factor.

focused on adults, in vitro and animal models, non-infectious arthritides or a few select cytokines. Of interest, we documented differential increases in cytokine levels for synovial fluid versus blood for cytokine groups with known intra-articular expression.[19 20] Levels of GCSF and the eotaxin group, for example, were significantly more elevated in the synovial fluid of cases compared with controls, but not in the serum, which is suggestive of intra-articular expression.[19 20]

Many cytokine levels in our synovial fluid samples were in range with previous reports, though a few differences were noted. Osiri *et al* described 23 adults with septic arthritis with median levels of TNF-α and IL-6 of 334.0 and 2185.2 pg/mL, respectively, compared with our values of 40.3 and 8294.3 pg/mL.[3] Steiner *et al* described a median synovial fluid level of IL-6 in adults with reactive or rheumatoid arthritis of 4500 pg/mL, corresponding to our median level of 8294.3 pg/mL, while median levels of interferon-γ (17 pg/mL) and TNF-α (157 pg/mL) were lower than this, but in a similar range to our findings (median levels of 49.4 and 40.3 pg/mL, respectively).[21]

Many of the elevated cytokines in our study are also elevated in non-infectious arthritides (eg, IL-6, IL-8, eotaxins, the CCL and CXC chemokine families), suggesting a role in intra-articular inflammation in general.[13 21] IL-6 elevation in synovial fluid (demonstrated in our study) is also associated with non-gonococcal septic arthritis in adults, reactive arthritis and *K. kingae* septic arthritis.[3 5]

Our study also documented increases in the synovial fluid levels of chemokines (CXC, eotaxin and CC groups). Elevations in the levels of these chemokines in the blood and synovial fluid of adults with non-infectious arthritides have been reported before,[13 19] though to our knowledge, this has not been described in children with septic arthritis.

Our study benefited from simultaneous collection of blood and synovial fluid and the use of a comparison cohort undergoing orthopedic surgery for non-infectious indications, thus helping to control for confounders associated with the timing of collection of specimens or the surgery itself. Given the complex interplay between cytokines, the commonly used classification into 'anti-inflammatory' and 'pro-inflammatory' groups was felt to

Table 2 Synovial fluid cytokine analysis in children with and without septic arthritis

	Control (pg/mL)			Case (pg/mL)			
Cytokine group	Mean	Median	Range	Mean	Median	Range	P value
CXC	833.2	979.3	254.4–1231.4	1273	1394.8	773.7–1528.8	0.0727
CCL	437.8	425.4	47.8–1020.4	1147.8	1179.9	584.4–1683.1	0.0727
Eotaxins	16.3	19	2.8–30.7	43.1	50.5	19.9–51.4	0.0283*
MIF	1241.2	803.1	143.5–4839.8	1942	1434	891.3–4008.5	0.3678
GCSF	15.7	16.3	6.3–27.2	36	31.1	22.0–59.7	0.0162*
IL-1	26.4	21.7	7.8–65.8	83.7	84.6	30.3–135.5	0.0162*
IL-10	18.6	19.2	4.3–39.3	149.8	45.8	23.5–484.0	0.0727
Interferon-γ	21.3	23	5.0–37.8	49.1	49.4	27.0–70.5	0.074
Common-γ side chain	16.1	17.7	2.8–26.1	103.3	101.2	57.3–153.7	0.0040*
IL with chemokine properties	87.2	73.5	21.4–145.6	4404.4	1720.7	229.6–13,946.4	0.0040*
IL-6	10.4	10.7	1.5–17.5	9857.3	8294.3	168.9–22,671.0	0.0040*
TNF-α	19.7	22	4.0–38.8	46.2	40.3	32.0–72.3	0.0160*

*Statistically significant; Mann-Whitney analysis used, with significance determined with the Benjamini-Hochberg correction (false discovery rate of 10%).
GCSF, granulocyte colony-stimulating factor; IL, interleukin; MIF, macrophage migration inhibitory factor; TNF, tumor necrosis factor.

be too simplistic, and our categorization of cytokines into 12 groups with related structure and/or function helped conceptualize results. Our study was most limited by a small sample size, which we attempted to address through the use of non-parametric statistical analysis and corrected significance thresholds. This did, however, prevent us from studying variables which may impact the intra-articular inflammatory response, such as age and the infecting pathogen. Indeed, it is likely that different pathogens may incite variable inflammatory responses, though study of this phenomenon would require much larger populations, as up to two-thirds of children with septic arthritis may not have a pathogen isolated.[22] The inability to isolate a pathogen from 3 of our cases is another limitation of our study, as this prevented us from definitively excluding non-infectious etiologies in these subjects. However, given the high Kocher scores for these subjects, as well as the prompt resolution of symptoms with antibiotics, we feel an infectious etiology was most likely.

Our results provide support for further study of serum and synovial fluid cytokine levels in larger cohorts with multiplex cytokine assays and newer sequencing technologies (eg, RNA-Seq). Such study may identify unique patterns of cytokine expression associated with different forms of infectious and non-infectious arthritides, better characterize the natural evolution of the intra-articular inflammatory response in septic arthritis to monitor disease progression and identify cytokines associated with articular damage which may be candidates for targeted suppression with monoclonal antibody therapy.

Acknowledgements We thank Laurie Wells, PhD, for her statistical support in this project.

Contributors All authors were involved in the conduct of the study and the creation of all drafts of the manuscript. ST was also involved in laboratory analyses, WD in data analysis, and SS, SP, KCH, NH and AP in specimen collection and consent.

Funding This study was funded by an institutional Signature Program in Child Health grant.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This project was approved by the Human Research Review Committee of the University of New Mexico School of Medicine (HRRC 17-414), with informed consent obtained from all subjects.

Provenance and peer review Not commissioned; externally peer reviewed.

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