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IL-1β, IL-6 and TNF-α in Synovial Fluid of Patients with Non-Gonococcal Septic Arthritis

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SUMMARY **Interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are the main proinflammatory cytokines responsible for the inflammatory process and cartilage destruction of inflammatory arthropathies. The present study sequentially measured the concentrations of these cytokines and their proportions of detectable levels in the synovial fluid (SF) of 23 patients with non-gonococcal (GC) septic arthritis before and after treatment. Persistently high concentrations and proportions of IL-6 and TNF-α) were found up to day 7 of treatment, while SF IL-1β concentration declined significantly after day 7 ($p = 0.036$). SF IL-1β and TNF-α correlated with each other significantly and with SF WBC counts ($p < 0.01$). Positive correlations between SF IL-1β concentration and joint effusion ($p < 0.01$) and between SF TNF-α concentration and joint tenderness ($p < 0.001$) were observed. SF IL-1β and TNF-α were significantly higher in patients with local complications of septic arthritis. In conclusion, high levels of IL-1β, IL-6 and TNF-α were detected in SF of patients with non-GC septic arthritis. Only IL-1β decreased significantly after day 7 of treatment, but IL-6 and TNF-α concentrations were persistently high. SF IL-1β and TNF-α may be useful in predicting the outcome and complications of patients with this disease.**

Interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are grouped as proinflammatory cytokines. These glycoprotein mediators, which can be produced by most nucleated cells, play a major role in inflammatory processes.[1,2] They can be detected in a variety of inflammatory joint diseases, both acute and chronic ones.[1,2] Extensive studies of cytokines have been performed in rheumatoid arthritis,[3-6] and some in other inflammatory arthropathies such as infectious arthritis,[3,5,7] and crystal-induced arthritis.[5]

In septic arthritis, once the microbes or microbial antigens enter the joint, they stimulate the release of proinflammatory cytokines. These cytokines are produced by mononuclear phagocytes residing in the joint.[1] The main effect of these cytokines is to induce inflammatory responses by mechanisms of recruiting neutrophils into the site of inflammation, stimulating chondrocyte production of collagenase, nitric oxide and prostaglandin E2, inhibiting collagen, proteoglycan and metalloproteinase inhibitor synthesis and triggering monocytes to produce more cytokines. The results are cartilage and bone damage as well as a repairing process with defective components, leading to secondary osteoarthritis.[8-10]

The major proinflammatory cytokines which stimulate joint inflammation and destruction in septic arthritis are IL-1, IL-6 and TNF-α. There were reports of high levels of these cytokines detected in synovial fluid (SF) of patients with septic arthritis which correlate

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with SF white blood cell count.[3,7] All of these reports, however, were cross-sectional studies and neither follow up after treatment nor correlation of these cytokines with clinical features were performed.

The objectives of this prospective study are to determine the concentrations of IL-1β, IL-6 and TNF-α in SF of patients with non-gonococcal (GC) septic arthritis and the correlation of measured cytokines with clinical features.

MATERIALS AND METHODS

Patients

Twenty three patients, aged 18 years or more, admitted to Chulalongkorn University Hospital from March 1996 to Feb 1997 were included in this study. All of the patients were diagnosed non-GC septic arthritis by clinical features of acute arthritis and positive culture for non-GC bacteria from synovial fluid and/or blood. Clinical manifestations included joint swelling (present/absent), joint effusion (present/absent), erythema around the affected joint (present/absent), joint tenderness (0 = no tenderness on palpation, 1 = tenderness on forceful palpation, 2 = tenderness on gentle palpation, 3 = tenderness on touching) and joint pain (0 = no pain, 1 = pain occurred on joint motion, 2 = intermittent pain occurred at rest, 3 = persistent pain) were recorded on day 0 (before commencing antibiotics), at 24 hours, 72 hours and 7 days after antibiotics were administered. Informed consent was obtained from all patients.

Collection of SF samples

Arthrocenteses of the affected joints were performed before and after treatment was initiated. The SF samples collected before treatment, at 24 hours, 72 hours and 7 days after treatment of each patient were centrifuged at 1,500 *g* for 10 minutes and the supernatants were stored at -70°C until assayed. The rest of the sample was analyzed for total WBC count, neutrophil percentage and sent for bacteriologic culture. Complete blood count and routine culture were also obtained on days 0 and 7.

Assays for cytokines

IL-1β and TNF-α were measured by enzyme-linked immunosorbent assay (ELISA) from Cellular Technology Institute, Otsuka Pharmaceutical, Co. Ltd., Japan. The lower limit of sensitivity of the assay was 6 pg/ml for IL-1β and 20 pg/ml for TNF-α. IL-6 was determined using ELISA kits from INCSTAR Corp., USA with the lower limit of sensitivity of 3 pg/ml. The method used for determination of cytokines was antibody-sandwich ELISA. In brief, anti-TNF-α monoclonal antibody was coated on the wells of ELISA plates. Standard TNF-α dilution series and SF samples were added to the antibody-coated wells. Then an enzyme conjugated second antibody was added to bind to TNF-α which was captured by the first antibody. Substrate solution was added to each well and the reaction was measured by spectophotometry. The assay procedures of SF IL-1β and IL-6 were similar except that anti IL-6 monoclonal antibody has already been coated on the wells. Negative controls for ELISA (no standard or sample added) were also tested. All samples were assayed for cytokines in duplicates. The cytokines concentrations (in pg/ml) were calculated in comparison with standards using the Delta-Soft program.

Statistical analyses

Median concentrations and proportions of detectable cytokines were described. The Pearson correlation coefficients between the cytokine concentrations and clinical indices of joint inflammation were calculated. Comparison of cytokine concentrations between clinically different groups was performed using non-parametric tests (Mann Whitney U test and Kruskal Wallis test). The significance level was determined at $p < 0.05$.

RESULTS

Twenty three adult patients were enrolled in this study. There were 11 males and 12 females. The mean age of the patients was 46.7 years, with a range of 18-77. Twenty-one patients (91.3%) had underlying medical diseases while 10 (43.5%) had underlying bone and joint diseases. The most affected joint was knee (18 cases, 78%) and most patients had monoarticular arthritis (22 cases, 95.7%). Right-sided joint inflammation was found more commonly than left-sided inflammation (56.5% vs 39%). Complications were found in 12 patients (52.2%) which included loculated joint effusion in 3 patients (13%); septicemia, periarticular cellulitis and post-infectious arthritis in 2 each (8.7%); and 1 patient (4.3%) in each of the followings: infective endocarditis, upper gastrintestinal bleeding and drug eruption. Three patients (13%) required surgical drainage of the infected joints. There were 2 deaths (8.6%) in our study. One

Figure 1a. SF IL-1 β concentrations and medians in non-GC septic arthritis.

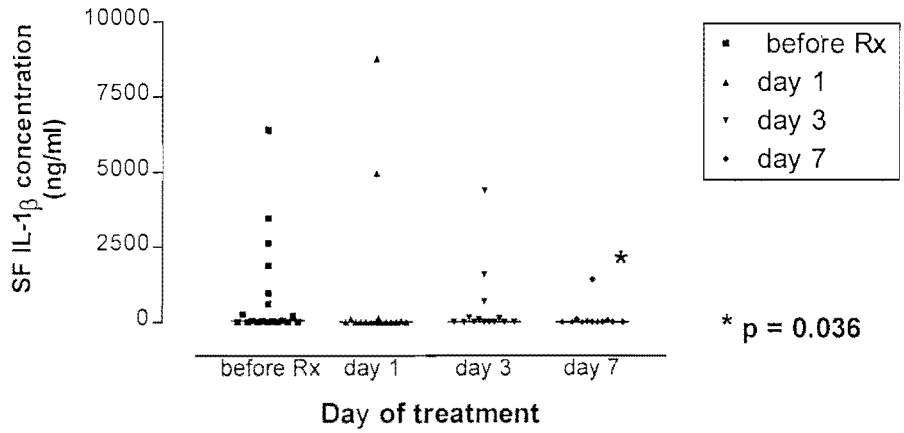

Figure 1b. SF IL- 6 concentrations and medians in non-GC septic arthritis.

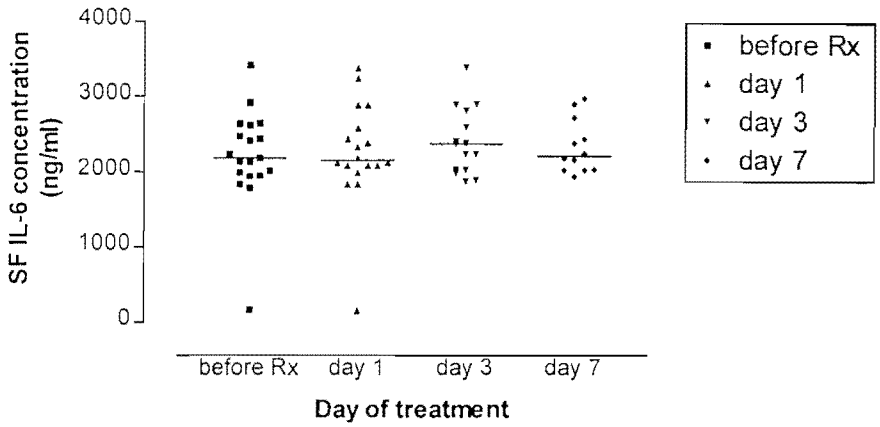

Figure 1c. SF TNF-α concentrations and medians in non-GC septic arthritis.

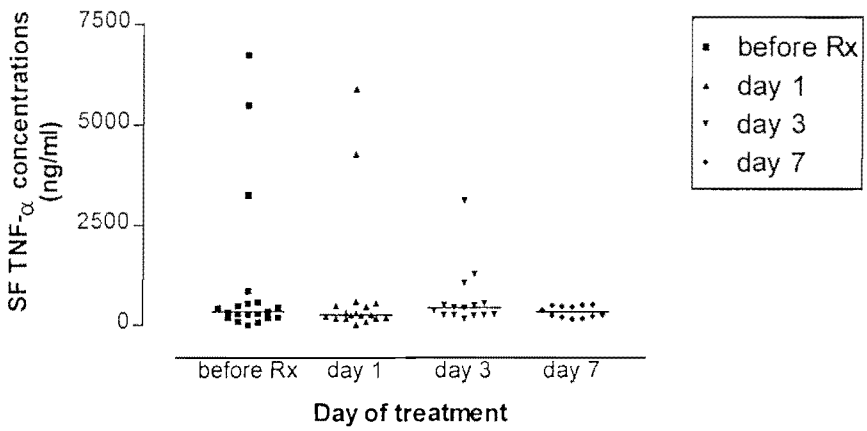

Fig. 1 SF IL-1β (a); IL-6 (b) and TNF-α (c) concentrations and medians in non-gonococcal septic arthritis.

had underlying alcoholic liver disease and died from upper gastrointestinal hemorrhage while the other had diabetes mellitus and died from septicemia. Demographic characteristics are summarized in Table 1.

Median SF WBC counts on day 0, at 24 hours, 72 hours and day 7 of treatment were 50,000, 40,000, 22,000 and 12,450 cells/mm^3, respectively while percentages of neutrophils were 93.7, 93.3, 88.2 and 81.8. There were significant differences between day 0 and day 7 in both parameters ($p < 0.05$).

SF gram stain was positive for bacteria in 11 patients (48%) before antibiotics were administered. Bacteriologic data from synovial fluid and blood cultures before starting of treatment are shown in Table 2. The most common organism was *Staphylococcus aureus* (9 patients; 39.1%). Streptococci were found in 8 patients (34.8%), while gram negative bacilli caused septic arthritis in 5 (21.5%).

IL-1β was detected (level > 6 pg/ml) in 68% of SF samples from patients before administration of antibiotics. The proportion of detectable IL-1β declined to 31% ($p < 0.05$), 43% and 33% at 24 hours, 72 hours and day 7 of treatment, respectively. IL-6 was detected in all SF samples throughout the study while TNF-α was detected (level > 20 pg /ml) in 95% of SF samples before and at 24 hours of treatment, and 100% at 72 hours and day 7 of treatment.

Median concentrations of IL-1β in SF were 49.8, 0, 0 and 0 pg/ml before and at days 1, 3 and 7 of treatment, respectively. Those of IL-6 were 2,185.2, 2,157.4, 2,377.9 and 2,215.5 pg/ml and TNF-α were 334.0, 252.4, 439.4 and 326.9 pg/

Table 1 General data of patients with non GC septic arthritis

Characteristics	Value (%) (n = 23)
Mean age ± SD (years)	46.7 ± 3.6
Age range (years)	18-77
Sex (M:F)	11:12 (47.8%:52.2%)
No. of patients with underlying medical disease	21 (91.3%)
No. of patients with underlying bone and joint disease	10 (43.5%)
No. of affected joints (one:three joints)	22 : 1 (95.7:4.3%)
No. of affected joints (knee:ankle)	18:5 (78:22%)
Site of affected joint (right:left:bilateral)	13:9:1 (56.5:39.1: 4.3%)
No. of patients with complication	12 (52.2%)
No. of patients requiring surgical intervention	3 (13.0%)
No. of dead patients	2 (8.7%)

Table 2 Bacteria cultured from SF and/or blood of 23 patients

Bacteria	Number of positive culture results (%) (n=23)			
	In SF only	In blood only	In SF and blood	Total
Staphylococcus aureus	6 (26.1)	2 (8.7)	1 (4.3)	9 (39.1)
Burkholderia pseudomallei	0	0	1 (4.3)	1 (4.3)
β-*Streptococcus* group A	2 (8.7)	0	1 (4.3)	3 (13.0)
Salmonella	2 (8.7)	0	0	2 (8.7)
Enterococcus fecalis	1 (4.3)	0	0	1 (4.3)
Other streptococci	2 (8.7)	2 (8.7)	1 (4.3)	5 (21.7)
Other gram negative bacilli	1 (4.3)	0	1 (4.3)	2 (8.7)
Total	14 (60.9)	4 (17.4)	5 (21.5)	23

ml. Scattergrams and medians are shown in Fig. 1. There was a significant difference in SF IL-1β on day 7 compared with day 0 ($p < 0.05$) while SF IL-6 and TNF-α did not change significantly throughout the study.

Correlations between SF IL-1β, IL-6 and TNF-α and clinical parameters

The IL-1β concentration in SF samples collected from patients on day 7 of antibiotic treatment correlated significantly with signs of joint effusion ($r = 0.662$, $p < 0.01$) and SF TNF-α on day 7 correlated positively with joint tenderness ($r = 0.802$, $p < 0.001$). No significant correlation was detected on the other days.

SF IL-1β and TNF-α concentrations of patients with local complications of septic arthritis (ie. loculated effusion, periarticular cellulitis) were significantly higher than those of patients without local complications ($p < 0.05$) only before and at 24 hours of treatment.

SF TNF-α concentrations before antibiotic therapy of patients who needed surgical intervention of affected joints (ie. arthroscopic lavage or open drainage) were also significantly higher than those of patients who responded to repeated joint aspiration ($p < 0.05$).

Correlations between SF IL-1β, IL-6 and TNF-α and laboratory parameters

Before starting antibiotic administration, SF IL-1β concentrations correlated significantly with SF TNF-α ($r = 0.74$, $p = 0.0002$) and also with SF WBC count ($r = 0.535$, $p < 0.01$).

SF TNF-α concentration correlated significantly with SF IL-1β on day 1 of treatment ($r = 0.846$, $p < 0.0001$) and with SF WBC count on day 3 of treatment ($r = 0.912$, $p < 0.0001$).

DISCUSSION

Clinical manifestations of patients with non-GC septic arthritis in this study were similar to previous reports.[11,12] Most had acute monoarticular arthritis as presenting symptoms. As in previous studies, *Staphylococcus aureus* was the most common causative organism cultured from infected SF.[11,12]

In septic arthritis, there is strong evidence that proinflammatory cytokines play a major role in the process of inflammation, cartilage and bone degradation.[1-3,13] The results of our study confirmed this information. Median concentrations and the proportions of detectable IL-1β, TNF-α and IL-6 in SF samples were high before treatment. Only IL-1β decreased significantly after starting antibiotic therapy while IL-6 and TNF-α were persistently high through day 7 of study. 31.6% of patients had zero levels of SF IL-1β pre-treatment that has not been reported elsewhere. These findings might be a result of the short half-life of these cytokines.[14,15]

The roles of IL-1β in septic arthritis are acute inflammation and destruction and impairment of the reparative process. IL-1β, which is strongly induced by bacteria and their products, stimulates infiltration of polymorphonuclear and mononuclear cells and the production of more proinflammatory cytokines and degradative enzymes.[2] It also inhibits chondrocyte proliferation as well as synthesis of collagen and proteoglycan.[16] Osteoclast activity is stimulated by IL-1β and results in osteoporosis.[17] Thus, the total effects of IL-1β on septic arthritis are acute, short term but profound, inflammation. These mechanisms can explain the finding of high concentrations of SF IL-1β before treatment and decreased levels after initiation of antibiotics. Persistent high concentrations and proportion of detectable TNF-α and IL-6 throughout the study can be described in different ways. TNF-α, although most of its activities overlap with those of IL-1β, is less active in causing cell accumulation in the joint, and all of these cells are mononuclear cells.[2] TNF-α can be continuously produced from stimulation of bacterial products.[2] The total effects of TNF-α are then more "subacute" and less severe than those of IL-1β.

IL-6 has many different effects on inflamed joints compared with IL-1β and TNF-α. Most of IL-6 activities produced proinflammatory effects as those of IL-1β and TNF-α and both cytokines can stimulate IL-6 secretion. IL-6 may act as a marker of inflammatory process without definite effects on the inflamed joints. However, IL-6 also has "good" effects including stimulation of tissue inhibitor of metalloproteinase (TIMP) release, blockade of metalloproteinase and reduction of lipopolysaccharide (LPS)-induced TNF expression in monocytes.[2,15] These activities may reduce inflammation and destruction of the infected joints.

Correlations between SF TNF-α and IL-1β were observed in this study. Each also correlated with SF WBC count as described in

previous study.[7] Clinical parameters of joint inflammation also correlated with these two cytokines. The findings of a significantly higher concentration of IL-1β and/or TNF-α in SF samples taken from patients with local complications of septic arthritis, and those who needed surgical intervention, may be a useful predictive marker for the early surgical management of septic arthritis to prevent further damage to the infected joints. There were 2 deaths in this study. Both patients had underlying medical illnesses (alcoholic liver cirrhosis and diabetes mellitus). No significant differences in SF proinflammatory cytokines were detected in these patients compared with the others.

In conclusion, the findings of our study might postulate that, in septic arthritis, IL-1β in SF plays a major role in acute joint inflammation and destruction, TNF-α has milder and more chronic effects whereas IL-6 may play a dichotomous role in both inflammation and protection from arthritis.

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REFERENCES

1. Dayer JM, Arend WP. Cytokines and growth factors. In: Kelley WN, Harris ED, Jr, Ruddy S, Sledge CB, eds. Textbook of rheumatology 5th ed. Philadelphia: WB Saunders, 1997; pp. 267-86.
2. Lotz M. Cytokines and their receptors. In: Koopman WJ, ed. Arthritis and allied conditions 13th ed. Baltimore: William & Wilkins, 1997; pp. 439-78.
3. Yoshida K, Kobayashi K, Yamagata N, *et al.* Inflammatory cytokines and enzymes in synovial fluid of patients with rheumatoid arthritis and other arthritides. Int Arch Allergy Immunol 1992; 98: 286-92.
4. Waage A, KaufmannC, Espevik T, *et al.* Interleukin-6 in synovial fluid from patients with arthritis. Clin Immunol Immunopathol 1989; 50: 394-8.
5. Houssiau FA, Devogelaer JP, Van Damm J, *et al.* Interleukin-6 in synovial fluid and serum of patients with RA. and other inflammatory arthritides. Arthritis Rheum 1988; 31: 784-8.
6. Thomas R, Carroll GJ. Reduction of leukocyte and interleukin-1 beta concentrations in the synovial fluid of rheumatoid arthritis patients treated with methotrexate. Arthritis Rheum 1993; 36: 1244-52.
7. Saez-Llorens X, Mustafa MM, Ramilo O, *et al.* Tumor necrosis factor α and interleukin-β in synovial fluid of infants and children with suppurative arthritis. AJDC 1990; 144: 353-6.
8. Oppenheim JJ, Ruscetti FW, Faltynek C. Cytokines. In: Stites DP, Terr AI, Parslow TG, eds. Basic and clinical immunology 8th ed. USA: Appleton & Lange, 1994; pp. 105-23.
9. Conrad LW, Van Voorhis WC. Review: nomenclature and biologic significance of cytokines involved in inflammation and the host immune response. J Infect Dis 1995; 175: 1573-80.
10. Arend WP, Dayer JM. Cytokines and cytokines inhibitors or antagonists in rheumatoid arthritis. Arthritis Rheum 1990; 33: 305-15.
11. Deesomchok U, Tumrasvin T. Clinical features of bacterial arthritis (nongonococcal arthritis). Chula Med J 1989; 32: 289-97.
12. Goldenberg DL, Reed JI. Bacterial arthritis. N Engl J Med 1985; 312:764-71.
13. Pettipher ER, Higgs GA, Henderson B. Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. Proc Natl Acad Sci USA 1986; 83: 8749-53.
14. Dinarello CA. Interleukin-1. In: Thomson A , ed. The cytokine handbook. 2nd ed. London: Academic Press, 1994; pp. 31-56.
15. Hirano T. Interleukin-6. In: Thomson A, ed. The cytokine handbook 2nd ed. London: Academic Press, 1994; pp. 145-68.
16. Chandrasekhar S, Harvey AK, Stack ST. Degradative and repair responses of cartilage to cytokines and growth factors occur via distinct pathways. Agents Actions 1993; 39 (suppl): 121-5.
17. Roodman GD. Role of cytokines in the regulation of bone resorption. Calcif Tissue Int 1993; 1(suppl 53): S94-S98.