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ORIGINAL ARTICLE

# Persistence of high levels of IL-6 and IL-8 in the cerebrospinal fluid of children with bacterial meningitis and prolonged fever

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***Objective*:** Changes in the concentration of inflammatory cytokines in the cerebrospinal fluid (CSF) and serum of patients with bacterial meningitis (BM) have been the objective of several studies mainly with respect to their prognostic value. The aim of our study was: 1. To investigate the serial changes of IL-1β, TNF-a, IL-6 and IL-8 in the CSF of children with BM during the first three days from the disease onset and 2. To correlate our findings with the disease complications and outcome.

***Methods:*** Thirty two children, hospitalised for BM during a 2 year and 4 month period, were studied. In 20 cases a causative agent was isolated (N. meningitides=9, H.influenzae=9, S. pneumoniae=2) while in 12 of them no pathogen was found. All patients were treated with ceftriaxone (100mg/kg/d IV for 7-10d) and dexamethasone (0,6mg/kg/d IV for 2d). The CSF and serum samples were obtained concomitantly on admission (1st), 12-24h later (2nd) and 52-72h after the 1st sample. Levels of IL-1β, TNF-a, IL-6 and IL-8 were assessed using the Quantikine Immunoassay Kit.

***Results*:** None of the cases was complicated by shock, none of the patients died and all were cured. 7/32 children presented with secondary fever and 5/32 with prolonged fever. In 4 out of these 5 patients subdural effusion was also present. The levels of all cytokines studied, were increased in the CSF and serum samples but they were significantly higher in the CSF. In the patients with prolonged fever (5) levels of IL-6 and especially of IL-8 in the CSF were persistently higher than those found in patients with secondary fever or early apyrexia, while in serum no difference was found. No correlation of cytokine levels and some other parameters studied (day of disease on admission, treatment prior to admission, causal agent of meningitis etc) was found.

***Conclusions:*** Prolonged fever with or without subdural effusion in patients with BM is related with persistence of high CSF levels of IL-6 and IL-8 for more than 3 days. This may be indicative of persistence of the inflammatory process elsewhere in the CNS and may need immunomodulatory therapy. *Hippokratia 2003, 7 (4): 177-181*

Bacterial meningitis (BM) has an unacceptable mortality rate and frequency of neurological sequellae despite the use of newer more effective antibiotics and hospitalization of the patients in well organized intensive care units [1-3]. This poor outcome was partly attributed to the induction of an excessive host immune response by bacterial cell wall products that resulted in an intrathecal overproduction of cytokines and other inflammatory mediators which exert a detrimental effect on the central nervous system. [1-6] On the basis of the above concept, changes in the concentration of inflammatory cytokines in the cerebrospinal fluid (CSF) and serum of patients with BM were studied, mainly with respect to their prognostic value. More specifically, the kinetics of TNF-a, IL-1β, IL-6 and IL-8 were studied during the first 24 or 48 hours after the initiation of the disease treatment. [7-12]

The aim of the study was to extend the investigation of changes of the above mentioned cytokines for a longer period of time and to correlate our findings with the complications and outcome of the disease.

## Patients and methods

The study was prospective and lasted for 2 years and 4 months (1995-1997). During this period, 59 patients with BM were hospitalized in the Department of Paediatrics of the Infectious Diseases Hospital of our city. All patients originated from the area of Northern Greece. Thirty two of them (13 males, 19 females), aged 3 months to 14 years, were included in the study after a written consent of the parents. Twenty seven children were not included in the study because lumbar punctures have not done according to the protocol, or there was no consent from the parents.

The diagnosis of bacterial meningitis was based on two or more of the following criteria: 1) typical clinical features, 2) findings in the CSF consistent with BM (pleiocytosis-neutrophiles, low glycose and high protein level), 3) isolation of a microorganism from the CSF

and/or blood culture, 4) demonstration of bacterial antigens by a rapid slide agglutination technique ("Slidex meningitis Kit" – BioMerieux Laboratory). Causes of bacterial meningitis were: N. meningitidis in 9, H. influenzae type b in 9 and S. pneumoniae in 2 cases. In 12 cases no pathogen was isolated. These cases fulfilled the first two criteria Thirteen out of 32 patients were admitted during the first day, 11 during the second and 8 during the third day of illness. As first day we considered the day of onset of fever (>38oC). In 22/32 patients an antibiotic had been given intravenously (IV) before admission: (ceftriaxone in 10 and one or more of the following in the remaining 12: ampicillin, amoxicillin/clavulanic, ceftazidime, cefuroxime, cefotaxime, chloramphenicol). Seventeen patients had received concurrently with the antibiotic one dose of prednisone or dexamethazone.

After admission, all patients were treated with the same regimen: ceftriaxone 100mg/kg/d IV for 7-10 days and dexamethasone 0,6mg/kg/d IV for 2 days. In 4/32 patients a change of the therapeutic regimen was done because of persistence of fever and mild pleiocytosis in CSF. In patients with prolonged fever a brain CT scan was done.

Three consecutive serum and CSF samples were taken for cytokine measurements and other laboratory investigations: On admission, just before the initiation of our therapeutic regimen (1st sample), 12-24 hours later (2nd sample) and 52-72 hours after the 1st sample was obtained (3rd sample).

Levels of IL-1β, TNF-a, IL-6 and IL-8 were assessed using the solid phase quantitative enzyme immunoassay (Quantikine R&D Systems supplied by British Biotechnology Ltd) according to the manufacturer's instructions. Measurements with the Quantikine Immunoassay kit reflect the total amounts of cytokines (free amounts plus cytokines bound to soluble receptors, if any are present). The limits of detection were 0.3, 7.5, 0.7 and 18pg/ml for IL1-β, TNF-a, IL-6 and IL-8 respectively and the normal values given for the method were: 0-2.7, 0-7.5, 0-13.6 and 0-30pg/ml respectively.

All patients had a neurological evaluation on discharge and during a 2 year follow-up.

**Statistical analysis**

The paired t-student test was used for comparison of the mean cytokine values between the 3 CSF and the corresponding serum samples. The chi-square contingency analysis and Pearson's correlation coefficients were applied for comparisons between groups and correlation of the cytokine concentration with certain parameters, such as day of illness on admission, causal agent of meningitis, marked pleiocytosis or protein and glycose concentrations.

**Results**

The main demographic and some clinical data of the patients on admission and during the course of BM are summarized in table 1. In general, the course of the disease was quite good in all patients. None of the cases was complicated by shock, no patient died and all were cured. Seven patients presented with secondary fever (due to diarrhoea) and 5/32 with prolonged fever (> 10 – 40 days) despite the clinical and laboratory improvement of BM (reduction of the number of CSF leukocytes from 950-10.000/$mm^3$ before treatment to 100 – 1100/$mm^3$ on the 10th day). A subdural effusion was detected by CT scan in 4/5 patients with prolonged fever. During the 2 year follow-up no patient presented with any neurological sequellae.

Results regarding the cytokine measurements in the 3 samples of CSF and serum are shown in table 2. All cytokines studied were found to be increased in the 3 CSF and serum samples but they were significantly higher in the CSF samples than in the corresponding serum ones (table 2, $p < 0.001$). Levels of cytokines in the CSF were subsequently decreasing in the 2nd and 3rd samples (Fig. 1).

In the patients with prolonged fever (5/32) or prolonged fever plus subdural effusion (4/5), levels of IL-6 and IL-8 were found to be higher than levels found in children with early apyrexia or with secondary fever. Moreover, levels of these two cytokines remained high up to the 3rd CSF sample (IL-6: 269,76 ± 209,14pg/ml, IL-8: 5378,28 ± 4654,67pg/ml). Due to small number of patients with prolonged fever (5) and secondary fever (7) statistical analysis could not provide significant possibility. However, mean values correlation of these cytokines leads to indicative tendencies. The findings are shown in figure 2.

In children who received antibiotics and steroids prior to admission, levels of IL-1b in the 1st and 2nd CSF samples were found to be higher than those found in children who had not received any treatment but the difference of the mean values between the two groups of patients was not significant ($p > 0.05$).

No correlation between cytokine levels in each CSF sample and some other parameters studied (day of illness on admission, causal agent of meningitis, marked pleiocytosis or protein and glucose concentrations in CSF), was found ($r \leq 0,5$).

**Discussion**

The main findings of this study were the significantly higher concentrations of TNF-a, IL-1β, IL-6 and IL-8 in the CSF than in serum of children with BM and the correlation of prolonged fever with persistence of high CSF levels of IL-6 and IL-8 for at least 72 hours after admission.

Previous studies have shown that levels of the inflammatory cytokines, especially of IL-6 and IL-8 in the CSF of patients with BM are high up to 48 hours after initiation of treatment. [13-17] These findings along with the observation that levels of the same cytokines in the

**Table 1.** Main demographical and some clinical data of the 32 children with bacterial meningitis

| No | Age (months) | Sex | Day of illness on admission* | Treatment prior to admission** | Causal agent | Complications during the disease course |
|---|---|---|---|---|---|---|
| 1 | 11 | F | 3 | NO | N. meningitidis | Secondary fever, diarrhoea |
| 2 | 10 | F | 4 | NO | H.influenzae | Subdural effusion, Prolonged fever*** |
| 3 | 48 | F | 2 | YES | N. meningitidis | |
| 4 | 24 | F | 1 | YES | H.influenzae | |
| 5 | 24 | M | 3 | YES | Unknown | |
| 6 | 24 | F | 2 | NO | Unknown | |
| 7 | 15 | M | 2 | YES | Unknown | |
| 8 | 16 | F | 2 | YES | H.influenzae | |
| 9 | 54 | F | 2 | YES | Unknown | |
| 10 | 38 | F | 1 | NO | N. meningitidis | |
| 11 | 36 | M | 3 | YES | H.influenzae | Secondary fever |
| 12 | 3 | M | 1 | YES | Unknown | |
| 13 | 10 | F | 1 | YES | Unknown | |
| 14 | 60 | M | 2 | YES | N. meningitidis | |
| 15 | 21 | M | 2 | YES | N. meningitidis | |
| 16 | 24 | F | 3 | NO | Unknown | |
| 17 | 8 | F | 3 | NO | H.influenzae | Subdural effusion, Prolonged fever |
| 18 | 84 | M | 1 | NO | Unknown | |
| 19 | 22 | F | 2 | YES | N. meningitidis | Transient arthritis |
| 20 | 8 | F | 3 | YES | H.influenzae | Secondary fever, diarrhoea (Rota Zyme test +) |
| 21 | 7 | M | 1 | YES | Unknown | Secondary fever, diarrhoea |
| 22 | 120 | M | 2 | YES | H.influenzae | |
| 23 | 5 | F | 1 | NO | N. meningitidis | Subdural effusion, Prolonged fever |
| 24 | 9 | F | 2 | YES | Unknown | Secondary fever, diarrhoea |
| 25 | 20 | M | 1 | NO | N. meningitidis | |
| 26 | 13 | M | 2 | YES | H.influenzae | Subdural effusion, Prolonged fever |
| 27 | 54 | F | 2 | YES | Unknown | |
| 28 | 18 | F | 3 | YES | H.influenzae | |
| 29 | 10 | F | 2 | YES | N. meningitidis | Secondary fever, diarrhoea |
| 30 | 16 | M | 3 | NO | S. pneumoniae | |
| 31 | 60 | F | 3 | YES | Unknown | Secondary fever |
| 32 | 10 | M | 3 | YES | S. pneumoniae | Prolonged fever |

* As 1st day of illness was considered the 1st day of fever's (>38°C) appearance.
** Treatment means administration of hymisynthetic penicillin or/and 3rd generation cephalosporin IV or/and corticosteroid IM/IV.
*** Prolonged fever: persistence of fever for more than 10 days despite the improvement of clinical condition and CSF findings.

**Table 2.** Mean values of the inflammatory cytokines in the cerebrospinal fluid (CSF) and serum of the patients studied.

| Cytokines (mean ±SD) | CSF | | | Serum | | |
|---|---|---|---|---|---|---|
| | 1st sample* | 2nd sample* | 3rd sample* | 1st sample | 2nd sample | 3rd sample |
| IL-1b[1] | 214.2± 141.6 | 137.3± 127.4 | 57.0±110.9 | 23.4±43.2 | 25.4±46,2 | 10.9±20.0 |
| TNF-a[2] | 608.2±574.8 | 229.8±248.5 | 77.0±80.0 | 28.0±28.5 | 25.8±24.8 | 20.1±19.6 |
| IL-6[3] | 411.3±97.3 | 403.9±82.8 | 164.4±182.3 | 238.5±166.8 | 174.8±172.5 | 63.7±120.7 |
| IL-8[4] | 6585.9±4311.2 | 5652.2±4893.5 | 2511.3±3272.3 | 267.0±576.5 | 57.3±59.7 | 30.0±20.7 |

1: Reference values 0.3-2.7 pg/mL, 2: Reference values 4.4-7.5 pg/mL, 3: Reference values 0.7-13.6 pg/mL, 4: Reference values10-30 pg/mL
*The difference in the mean values of the 3 consecutive samples between CSF and serum was highly significant ($p<0.001$)

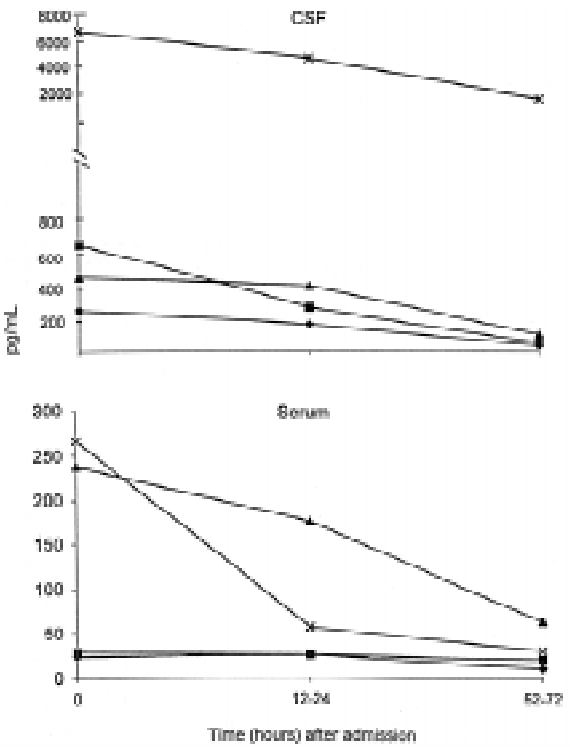


**Fig. 1.** Mean values of the cytokines (pg/ml) studied in the 3 consecutive cerebrospinal fluid (CSF) and serum samples in patients with purulent meningitis IL-1b (♦), TNF-a ( ■ ), IL-6 (▲ ), IL-8 ( X ).

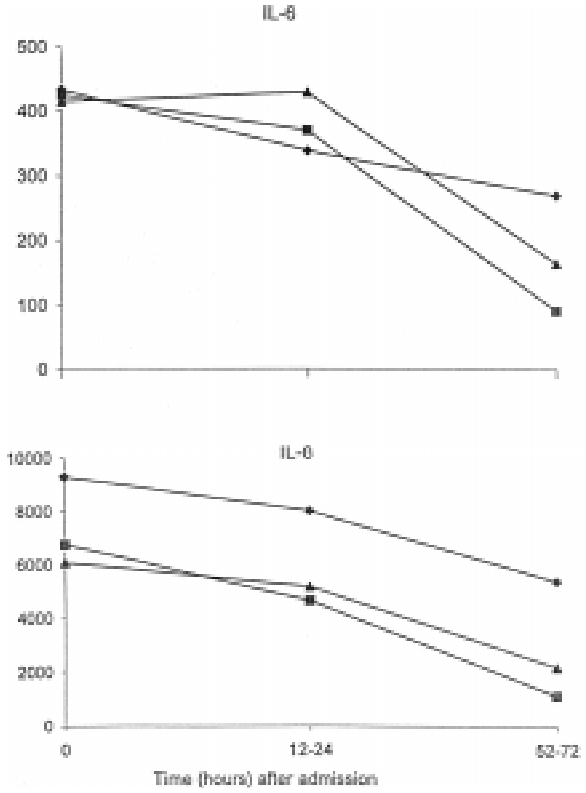


**Fig. 2.** Mean IL-6 and IL-8 levels (pg/ml) in the 3 consecutive cerebrospinal fluid samples (CSF) in patients with prolonged fever ( ♦ ), secondary fever ( ■ ) and fever of less than a 3-day duration (▲).

serum are much lower, led to the concept that the production of these cytokines is local as a result of an inflammatory process triggered by bacterial cell wall and membrane elements.[18] Findings of our study support this concept. Furthermore, they indicate that the mechanism of the cytokine synthesis may work for a longer period of time, namely, for at least 72 hours after the initiation of treatment.

High serum levels of inflammatory cytokines TNF-a and IL-6 in children with meningococcal septicaemia and shock have been correlated with high mortality rate. Furthermore, high CSF levels of IL-1β (> 500pg/ml) in children with BM, especially due to Hib, have been correlated with neurological sequellae [7,10,19,20]. Our data are consistent with these findings. None of our patients had such high serum levels of TNF-a, IL-Iβ and IL-6 and none developed shock or died. Also, no patient had CSF levels of IL-1β higher than 500pg/ml and none presented with any neurological sequellae during the 2 year follow-up period.

Levels and kinetics of IL-8 in the CSF of children with BM have been studied much less than the other three cytokines. Mastroianni and Paoletti found increased CSF levels of IL-8 in bacterial and tuberculus meningitis but not in aseptic meningitis.[14] They demonstrated a rapid decrease of IL-8 levels in children with bacterial meningitis, whereas they remained high for a longer period of time in children with tuberculous meningitis, probably due to the continuation of the inflammatory process in the latter. In our study, CSF levels of all cytokines measured were increased on admission and had a staging decline at the following 3 days without reaching normal limits. In particular, levels of IL-6 and IL-8 remained high for at least 72 hours in 5/32 children who displayed prolonged fever. In these children we, like Mastroianni and Paoletti, did not find significant correlation of the persistently high IL-8 levels with marked leukocytosis in the CSF (r = 0,32).

A lack of correlation of persistently high levels of IL-6 and IL-8 with the other parameters studied (treatment prior to admission, day of disease on admission, causal pathogen and protein and glucose CSF concentrations) has been also reported by previous investigators. [19-21]

Findings of this study indicate the following: Prolonged fever developing in some of the patients with BM is related with persistence of high CSF levels of IL-6 and IL-8, for more than 3 days. The coexistence of subdural effusion in most of such patients (4/5 in our material) suggests that the inflammatory process is going on elsewhere in the CNS and that its cessation may need immunomodulatory treatment.

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