Interleukin-8 in Cerebrospinal Fluid from Patients with Septic and Aseptic Meningitis

C. Østergaard[1,2*], T.L. Benfield[2], F. Sellebjerg[3], G. Kronborg[4], N. Lohse[5], J.D. Lundgren[2]

Using a monoclonal antibody enzyme immunoassay, the concentration of interleukin-8 (IL-8) in cerebrospinal fluid (CSF) from 52 patients suspected of having meningitis was studied. The CSF IL-8 concentration was significantly higher in septic meningitis of known and unknown etiology than in aseptic meningitis and significantly higher in aseptic meningitis than in patients without meningitis. The CSF levels of IL-8 correlated with the levels of tumor necrosis factor-α, leukocyte count, neutrophil count, protein level, CSF/blood glucose ratio, and the number of days patients were hospitalized. The IL-8 values used to distinguish septic from aseptic meningitis, at a cut-off point of 3.00 μg/l, showed a sensitivity of 81%, a specificity of 92%, and a positive predictive value of 96%. The results suggest that determining IL-8 levels may be useful in the differential diagnosis of meningitis.

Despite therapy with potent antibiotics and advances in intensive care technology, septic meningitis remains a significant cause of morbidity and mortality (1). Cytokines appear to play an important role in the pathogenesis of infections. Elevated levels of the cytokines, tumor necrosis factor-α (TNFα), interleukin-1-β (IL-1β), and interleukin-6 (IL-6) have previously been detected in cerebrospinal fluid (CSF) of patients with meningitis (2–5).

Table 1: Demographic and clinical characteristics of patients. All values are medians (95% confidence interval).

	Septic meningitis of known etiology	Septic meningitis of unknown etiology	Aseptic meningitis	Nonmeningitis
No. of patients	15	16	13	8
Age in years (range)	32 (14–53)	26 (8–38)	27 (20–32)	12 (3–78)
IL-8 (μg/l)	10 (7.7–95)	6 (2.6–14.5)	1.7 (0.6–2.5)	0.03 (0.03–0.12)
TNF-α (ng/l)	296 (0–2,980)	0 (0–0)	0 (0–0)	0 (0–0)
CSF (leukocytes x10^6/l)	2,508 (163–6,769)	475 (143–4,365)	71 (21–256)	2 (1–5)
Neutrophils (CSF x 10^6/l)	2,031 (91–5,560)	416 (128–3,404)	26 (0–63)	0.1 (0–2)
CSF glucose (mmol/l)	2.5 (1.1–4.2)	3.3 (2–4)	3.3 (3–3.7)	3.8 (3.4–4.3)
CSF/blood glucose	0.28 (0.01–0.49)	0.59 (0.33–0.69)	0.52 (0.49–0.67)	0.62 (0.52–0.76)
CSF protein (g/l)	1.9 (1.5–3.8)	0.8 (0.6–2.8)	0.5 (0.2–1.1)	0.3 (0.1–0.9)
Serum leukocytes (x10^9/l)	23.2 (13.1–25.3)	13.3 (8.7–17.3)	8.3 (7.0–10.6)	12.4 (6.5–26.6)
Back rigidity (%)	75	100	85	43
Decreased sensorium (%)	56	31	0	0
Days in hospital	12 (8–18)	10.5 (8–12)	3.4 (3–5)	4 (1–29)
Assisted ventilation (%)	56	6	0	0
Discharge to home (%)	87	94	100	100
Fatal outcome (%)	6	6	0	0

Interleukin-8 (IL-8) is a cytokine of the chemokine family and is produced by a variety of cells, including monocytes and endothelial cells, in response to bacterial lipopolysaccharide, TNFα, and IL-1β (6, 7). IL-8 is a potent inducer of neutrophil chemotaxis. It upregulates several neutrophil and endothelial adhesion molecules and induces transendothelial neutrophil migration (8, 9).

Septic meningitis, in contrast to viral meningitis, is characterized by the occurrence of a large number of neutrophils in CSF. The aim of the present study was to investigate the level of IL-8 in CSF and its utility in the differential diagnosis of meningitis.

Patients and Methods. Samples of CSF were obtained by lumbar puncture from 52 patients clin-

[1]Department of Clinical Microbiology, Statens Seruminstitut, Artillerivej 5, DK 2300 Copenhagen S, Denmark.
[2]Department of Infectious Diseases, University of Copenhagen, Hvidovre Hospital, 2650 Hvidovre, Denmark.
[3]Department of Neurology, University of Copenhagen, Glostrup Hospital, 2600 Glostrup, Denmark.
[4]Department of Medicine, University of Copenhagen, Gentofte Hospital, 2900 Gentofte, Denmark.
[5]Department of Dermatology, University of Aarhus, Marselisborg Hospital, 8000 Aarhus, Denmark.

ically suspected of having meningitis on admission (29 females and 23 males between the ages of 1 and 87 years). All patients with available CSF samples taken at admission were included. Based on clinical, microbiological, and biochemical characterization, the patients were divided into four groups. Group I comprised 15 patients with septic meningitis of known etiology (pleocytosis, positive CSF and/or blood cultures, or significant increases in antibody titers against *Neisseria meningitidis*). Six cases were due to *Neisseria meningitidis*, four to *Streptococcus pneumoniae*, four to *Haemophilus influenzae*, and one to *Staphylococcus aureus*. Group II comprised 16 patients with septic meningitis of unknown etiology (i.e., negative CSF and blood cultures, pleocytosis with > 80% neutrophils, a quick response to therapy with ampicillin in combination with ceftriaxone or netilmicin), and exclusion of other etiologies. Group III comprised 13 patients with aseptic meningitis (pleocytosis with a predominance of mononuclear cells and with full recovery without antibiotic treatment). Group IV comprised eight patients suspected of meningitis but without evidence of meningitis (i.e., no CSF pleocytosis), including two with meningococcemia, one with acute tonsillitis, three with fever of unknown origin, one with cystitis, and one with torticollis.

As a control, we also measured IL-8 levels in CSF from ten patients with various noninfectious diseases (e.g., headache, lower back pain, neuropathy). Samples of CSF were analyzed by routine laboratory methods including cell counts, glucose, and total protein determinations. Remaining CSF was centrifuged and the supernatants were stored at −20°C until subsequent assay of IL-8 and TNFα. Interleukin-8 and TNFα were measured by enzyme immunoassay as previously described (10, 11). The detection limits were 0.01 μg/l and 0.09 μg/l, respectively.

Statistical analysis was performed using the nonparametric Kruskal-Wallis and Mann-Whitney tests and the Spearman rank correlation test. When appropriate, Bonferroni corrections were used to compensate for multiple comparisons.

Results and Discussion. The concentrations of IL-8, TNFα, and clinical and routine laboratory data are shown in Table 1. All values are given as medians with 95% confidence intervals. Interleukin-8 was detected in CSF of all patients (Figure 1).

Interleukin-8 levels in CSF differed significantly between the four groups suspected of meningitis (Kruskal-Wallis; $p < 0.00001$). After pairwise

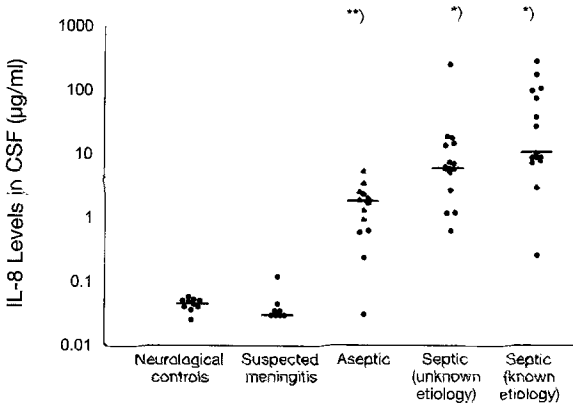

Figure 1: IL-8 levels in CSF (μg/ml) of patients with suspected or confirmed meningitis. *), $p < 0.05$ compared to other groups; **), $p < 0.05$ compared to nonmeningitis patients and controls. Bar indicates median.

comparisons of the levels of IL-8 (Mann-Whitney tests with Bonferroni correction for 6 comparisons), significant differences were observed between patients with septic meningitis of known and unknown etiologies when compared to patients with aseptic meningitis ($p < 0.0003$ and $p = 0.003$, respectively) as well as to patients suspected of having but without meningitis (both $p < 0.003$). Moreover, patients with aseptic meningitis had higher levels of IL-8 in CSF than patients without meningitis ($p = 0.005$). In contrast, there was no significant difference between patients with confirmed septic meningitis and septic meningitis of unknown etiology ($p = 0.18$).

Patients with impaired consciousness had significantly higher levels of IL-8 in CSF than conscious patients (15.6 μg/l, 5.7–17.1 vs. 2.00 μg/l, 0.92–5.30; $p = 0.003$). Patients given assisted ventilation therapy had significantly higher levels of IL-8 in CSF than patients not receiving such therapy (21.9 μg/l, 7.1–17.1 vs. 2.30 μg/l, 0.92–5.70; $p = 0.003$). There were no significant differences in the CSF IL-8 levels between patients with or without back rigidity, between patients who died or survived, or between patients discharged to their homes or transferred to other hospital departments.

There were significant correlations between CSF levels of IL-8 and TNFα ($n = 51$, $r = 0.722$, $p < 0.00001$), IL-8 and CSF leukocyte count ($n = 49$, $r = 0.625$, $p < 0.00001$), IL-8 and CSF neutrophil count ($n = 40$, $r = 0.516$, $p < 0.0007$), IL-8 and total protein level ($n = 45$, $r = 0.783$, $p < 0.00001$), IL-8 and CSF/blood glucose ratio ($n = 40$, $r = 0.583$, $p = 0.0008$), and IL-8 and the number of

days of hospitalization ($n = 51$, $r = 0.483$, $p = 0.0003$), while there was no correlation between IL-8 level and the number of mononuclear cells in CSF ($n = 40$, $r = 0.136$, $p = 0.4$). Within each of the four groups, there was no significant association between IL-8 and any of the variables listed above, except for a significant correlation between IL-8 and TNFα in patients with septic meningitis ($n = 31$, $r = 0.767$, $p < 0.00001$).

Others have described CSF levels of IL-8 in patients with meningitis. A previous study of children by Seki et al. (12), including seven with septic meningitis, found CSF IL-8 levels ranging from 0.066 to 0.860 μg/l in those with septic meningitis, whereas IL-8 was always below the detection limit of 0.03 μg/l in patients with aseptic meningitis or without meningitis. This study is at variance with our data. We found highly elevated IL-8 levels in septic meningitis and to a lesser degree in aseptic meningitis. In patients without meningitis, the levels were near the detection limit of the assay. The discrepancy between the study of Seki et al. (12) and ours may be due to improved sensitivity of our assay to detect IL-8 as well as the broader and larger consecutive patient population, including adults, in our study. Thus, we believe that determination of CSF IL-8 levels may distinguish septic from aseptic meningitis and aseptic meningitis from patients without meningitis.

In meningococcal disease, Halstensen et al. (13) found IL-8 levels from 0.10–50.3 μg/l in CSF of 19 patients with meningitis. They did not, however, find any significant difference between patients with or without meningitis. In our study there were eight patients with meningococcal disease, six with meningitis and highly elevated levels of IL-8 (> 8.6 μg/l), and two without meningitis and low levels of IL-8 (< 0.120 μg/l). Our study and that of Halstensen et al. (13) are, however, not comparable because of the noted differences in patient selection and the low number of patients with only septicemia in our study.

We found highly increased levels of IL-8 in CSF of patients with septic meningitis. Septic meningitis is defined by a large number of neutrophils in CSF, and IL-8 is one of the most potent inducers of neutrophil chemotaxis. Indeed, injection of IL-8 into the hippocampus resulted in a neutrophil inflammatory response in the ventricular system, choroid plexus, and meninges (14), suggesting an important role for IL-8 in the induction of neutrophil pleocytosis. IL-8 production is stimulated by cytokines such as TNFα and IL-1β, which are known to be elevated in septic meningitis. This may, indeed, have been the case in some of our patients since we found a significant correlation between IL-8 levels and TNFα levels in patients with septic meningitis.

The differences in IL-8 levels within the groups could be due to individual differences in the immunological response or to differences in the duration of symptoms on admission to hospital. The release of IL-8 could possibly precede neutrophil pleocytosis, as previously reported for TNFα, IL-1β, and IL-6 (2). The temporal relationship between the intrathecal release of IL-8, proinflammatory cytokines, and other laboratory findings in septic meningitis, however, remain to be defined.

As expected, we found that various parameters used in routine clinical practice that distinguish septic from aseptic meningitis (i.e., pleocytosis, decreased CSF/blood glucose ratio) correlated with CSF levels of IL-8 if all patients were included in the analysis, whereas no significant correlation could be detected if the groups were studied separately. This underlines that IL-8 levels can distinguish patients with septic meningitis from patients with aseptic meningitis or without meningitis.

We assessed the use of CSF IL-8 for diagnostic purposes to distinguish septic meningitis from aseptic meningitis. Employing a cut-off point of 3.00 μg/l or greater, the sensitivity was 81%, the specificity was 92%, and the positive predictive value was 96%. To distinguish aseptic meningitis from nonmeningitis using a cut-off point of 0.20 μg/l or more, the sensitivity was 92%, the specificity was 100%, and the positive predictive value was 100%. In comparison, using TNFα levels, we found a sensitivity of 34% with a specificity and a predictive value of 100%. This is most likely due to the low sensitivity of our TNFα assay. Others have reported markedly higher sensitivities, specificities, and positive predictive values of TNFα and IL-1β (> 75% for all) in the context of meningitis (3, 4, 15). Our results indicate that measurement of IL-8 may be helpful as an additional marker in distinguishing different forms of meningitis.

References

1. Quangliarello V, Scheld WM: Bacterial meningitis: pathogenesis, pathophysiology, and progress. New England Journal of Medicine 1992, 327: 864–872.
2. Waage A, Halstensen A, Shalby R, Brandtzaeg P, Kierulf P, Espevik T: Local production of tumor necrosis factor α, interleukin 1, and interleukin 6 in meningococcal

meningitis. Journal of Experimental Medicine 1989, 170: 1859–1867.
3. Ramilo O, Mustafa MM, Porter J, Saez-Llorens X, Mertsola J, Olsen KD, Luby JP, Beutler B, McCracken GH: Detection of interleukin 1β but not tumor necrosis factor-α in cerebrospinal fluid of children with aseptic meningitis. American Journal of Diseases of Children 1990, 144: 349–352.
4. Lopez-Cortes LF, Cruz-Ruiz M, Gomez-Mateos J, Jimenez-Hernandez D, Palomino J, Jimenez E: Measurement of levels of tumor necrosis factor-α and interleukin-1β in the CSF of patients with meningitis of different etiologies: utility in the differential diagnosis. Clinical Infectious Diseases 1993, 16: 534–539.
5. Chavanet P, Bonnotte B, Guiguet M, Zeller GV, Solary E, Maurice L, Casanovas O, Caillot D, Waldner A, Kisterman JP, Portier H: High concentrations of intrathecal interleukin-6 in human bacterial and nonbacterial meningitis. Journal of Infectious Diseases 1992, 166: 428–431.
6. Strieter RM, Kunkel SL, Showell HJ, Remick OG, Phan SH, Ward PA, Marks RM: Endothelial cell gene expression of a neutrophil chemotactic factor by TNF-α, LPS, and IL-1β. Science 1989, 243: 1467–1469.
7. Baggiolini M, Walz A, Kunkel SL: Neutrophil-activating peptide1/interleukin 8, a novel cytokine that activates neutrophils. Journal of Clinical Investigation 1989, 84: 1045–1049.
8. Huber AR, Kunkel SL, Todd RF, Weiss SJ: Regulation of transendothelial neutrophil migration by endogenous interleukin-8. Science 1991, 254: 99–102.
9. Smith WB, Gamble JR, Clark-Lewis I, Vadas MA: Interleukin-8 induces neutrophil transendothelial migration. Immunology 1991, 72: 65–72.
10. Ko Y, Mukaida N, Panyutich A, Voitenok NN, Matsushima K, Kaway T, Kashara T: A sensitive enzyme-linked immunosorbent assay for human interleukin-8. Journal of Immunological Methods 1992, 149: 227.
11. Kronborg G, Fomsgaard A, Shand GH, Lanng S, Hansen MB, Pressler T, Høiby N: TNF-α and immune complexes in sputum and serum from patients with cystic fibrosis and chronic *Pseudomonas aeruginosa* lung infection. Immunology & Infectious Diseases 1992, 2: 171–177.
12. Seki T, Joh K, Oh-Ishi T: Augmented production of interleukin-8 in cerebrospinal fluid in bacterial meningitis. Immunology 1993, 80: 333–335.
13. Halstensen A, Ceska M, Brandtzæg P, Redl H, Næss A, Waage A: Interleukin-8 in serum and cerebrospinal fluid from patients with meningococcal disease. Journal of Infectious Diseases 1993, 167: 471–475.
14. Andersson PB, Perry VH, Gordon S: Intracerebral injection of proinflammatory cytokines or leukocyte chemotaxins induces minimal myelomonocytic cell recruitment to the parenchyma of the central nervous system. Journal of Experimental Medicine 1992, 176: 255–259.
15. Roine I, Foncea LM, Cofre J, Ledermann W, Peltola H: Serum c-reactive protein vs. tumor necrosis factor alpha and interleukin 1 beta of the cerebrospinal fluid in diagnosis of bacterial meningitis with low cerebrospinal fluid cell count. Pediatric Infectious Disease Journal 1992, 11: 1057–1058.

Failure to Detect *Mycoplasma fermentans*, *Mycoplasma penetrans*, or *Mycoplasma pirum* in the Urethra of Patients with Acute Nongonococcal Urethritis

T. Deguchi[1,2], C.B. Gilroy[1], D. Taylor-Robinson[1]*

Urethral swab specimens collected from 108 male Japanese patients with acute nongonococcal urethritis (NGU) and from 50 Japanese men without NGU were examined for the presence of *Mycoplasma fermentans*, *Mycoplasma penetrans*, and *Mycoplasma pirum* by means of polymerase chain reaction–based assays. These mycoplasmas were not detected in any of the specimens, which suggests that they are unlikely to have a pathogenic role in acute NGU.

Chlamydia trachomatis is a cause of acute nongonococcal urethritis (NGU) in men, its pathogenicity in up to 50% of cases being well established (1). In chlamydia-negative NGU, however, the microbiological aetiology is not so well understood. There is some evidence for the role of *Ureaplasma urealyticum*, but the proportion of cases that might be attributable to this pathogen is undefined and there has never been any evidence that *Mycoplasma hominis* plays a significant part (2). Recently, however, *Mycoplasma genitalium* has been detected by means of polymerase chain reaction (PCR) assays significantly more often in urethral specimens from patients with acute NGU than in those from subjects without urethritis (3–5). Furthermore, this mycoplasma has been shown to produce urethritis in subhuman primates inoculated intraurethrally (6). The various results have suggested that this mycoplasma is a possible cause of NGU. Despite this, *Mycoplasma genitalium* has been detected in the urethra of only about 20% of patients with acute NGU (3–5), so that a substantial proportion of patients

[1] MRC Sexually Transmitted Diseases Research Group, The Jefferiss Trust Laboratories, Imperial College School of Medicine at St Mary's, Paddington, London W2 1NY, UK.
[2] Department of Urology, Gifu University School of Medicine, Gifu, Japan.