Cerebrospinal fluid interleukin-6 and its diagnostic value in the investigation of meningitis

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SUMMARY. We examined the measurement and the diagnostic value of cerebrospinal fluid interleukin-6 (CSF IL-6) in meningitis. The cytokine was measured by bioassay (B9 hybridoma cell line) and by immunoassay (in-house radioimmunoassay). We compared the diagnostic value of CSF IL-6 determination with that of other biochemical markers of meningitis.

Although there was significant correlation between bioactive and immunoactive IL-6 (r = 0.724, P < 0.01), results were frequently different with biological/immunological ratios ranging from 0.2 to 24.3 (mean 4.6). Gel permeation chromatography suggested that the discrepancy in biological and immunological activities was not due to molecular heterogeneity, but may be explained by the presence of a synergistic factor. Interleukin-6 concentration was markedly elevated in CSF from most patients with bacterial meningitis compared to patients with viral meningitis and those without evidence of infection. However, low IL-6 levels by radioimmunoassay did not exclude bacterial meningitis (sensitivity 86%). CSF total protein and CSF glucose were significantly different between all three groups, but there was no significant difference in lactate concentration between virally infected and normal CSF, both of which had lower lactate concentrations than those in bacterial infection.

CSF IL-6 measurement had greater sensitivity, specificity and predictive value than these other biochemical markers, and hence a rapid assay for IL-6 in CSF may contribute to the early diagnosis of bacterial infection.

Additional key phrases: cytokines; bioassay; immunoassay; inflammation

The diagnosis of meningitis relies upon biochemical and microbiological analysis of cerebrospinal fluid (CSF). Increased concentrations of total protein may be due to altered permeability of the blood brain barrier, intrathecal synthesis of immunoglobulins by activated lymphocytes, and/or the presence of bacterial proteins. Decreased CSF glucose concentration is suggestive of bacterial infection or the presence of neoplasm, whereas increased CSF lactate concentration is observed in bacterial meningitis.1,2 Despite the lack of specificity and sensitivity reported for these analytes, their measurement is widely used.3

A recent tentative marker of inflammation is interleukin 6 (IL-6). It is one of the inflammation-associated cytokines,4 previously known as B-cell stimulating factor, β-2 interferon, hybridoma/plasmacytoma growth factor and hepatocyte-stimulating factor. However, molecular cloning studies have shown that all of these molecules are identical.5 The discrepancy in nomenclature and delay in identification of this cytokine is due to the varying molecular weights reported (21–30 kDa). These differences may be attributed to post-translation modifications such as N- and O-linked glycosylation and phosphorylation6 or to cleavage or aggregation of the monomeric form of the cytokine.7 It is possible that molecular heterogeneity and/or alterations in glycosylation pattern may lead to changes in the biological and immunological activity of the cytokine.

IL-6 is produced by many cells including activated macrophages, lymphocytes and fibroblasts,
and the synthesis and release of the cytokine is stimulated by factors such as trauma, bacterial lipopolysaccharide, viruses and other cytokines, e.g. interleukin-1 and tumour necrosis factor (TNF; for review see reference 8). IL-6 is also a mediator of the systemic acute phase response, and circulating levels have been shown to be increased in bacterial infection. Moreover, IL-6 concentration increases in tissue damage and following various surgical procedures, the magnitude of the increase correlating with the severity of the insult. On the other hand, glucocorticoids inhibit IL-6 synthesis and IL-4 has been shown to antagonize the effects of IL-6, most likely due to the suppression of induction of endogenous IL-6 synthesis.

IL-6 has been detected in CSF and high concentrations were seen in patients with bacterial and viral meningitis. However, the diagnostic value of CSF IL-6 remains to be established.

In this study, the measurement of CSF IL-6 was examined, using two different assays (a bioassay and an immunoassay). We also determined IL-6 concentrations in CSF from patients being investigated for meningitis to determine whether CSF IL-6 correlates with other biochemical indices of inflammation and its diagnostic value in the investigation of meningitis.

MATERIALS AND METHODS

Patients' samples: CSF (n = 126) and serum samples (n = 50) were obtained from patients being investigated for meningitis admitted to the Royal Liverpool University Hospital. CSF samples (n = 115) were obtained from patients with bacterial meningitis from Malawi (Southern Central Africa), and from patients with viral meningitis (n = 5) from the National Hospital for Neurology and Neurosurgery, London, UK. Samples were taken at the initial presentation with the disease.

Microbiological analysis of the samples from Malawi was performed in the microbiology laboratory Kamuzu Central Hospital (Lilongwe, Malawi) as part of a project undertaken by one of us (CAH). These samples were frozen at −20°C within 2 h of sampling and transported frozen to Liverpool for analysis of glucose, lactate, protein and interleukin-6.

Reagents

CSF total protein assay reagents (Biotrol) were obtained from Apex Diagnostics (Hailsham, UK). CSF and serum glucose and CSF lactate assay reagents were obtained from Beckman (High Wycombe, UK). IL-6 international reference preparation (88/514), recombinant human IL-6, and goat anti-human IL-6 antiserum were obtained from Dr S. Poole, National Institute for Biological Standards and Control (NIBSC), (South Mimms, UK). Cellulose-coated donkey anti-goat antiserum (Sac-Cell) was obtained from IDS Ltd (Boldon, UK). Gel filtration molecular weight markers were obtained from Sigma Chemical Company (Poole, UK).

Biochemical analysis of CSF and serum

CSF total protein was determined by the Pyrogallol Red method, CSF and plasma glucose were determined by the hexokinase method, CSF lactate levels were determined by the lactate dehydrogenase method. All of the above assays were performed using a Beckman CX5 auto analyser.

Determination of CSF and serum IL-6

Immunoreactive IL-6. CSF IL-6 was determined using an in-house radioimmunoassay. IL-6 standard, CSF and serum samples (100 µL) were incubated with 200 µL phosphate-buffered saline containing 10 g/L bovine serum albumin (assay buffer) and 100 µL goat anti-IL-6 antiserum (diluted 1:400 000 in assay buffer) for 24 h at 4°C. 125I-labelled IL-6 (10 000 cpm/100 µL) was added and the mixture was incubated for 72 h at 4°C. Donkey anti-goat antiserum (SacCel) (100 µL) was added and the mixture was centrifuged after 30 min incubation. The precipitated pellet was washed twice in chilled assay buffer (2 mL). The radioactivity was counted and unknown IL-6 concentrations were determined from a log/logit plot.

Intra-assay imprecision (coefficient of variation, CV) was 6·1% at 0·2 ng/mL and 3·3% at 3·0 ng/mL, whereas, inter-assay CVs were 4·3% at 0·2 ng/mL and 11·7% at 3·0 ng/mL. The detection limit of the assay was 0·04 ng/mL.

Bioactive IL-6. Bioactive CSF IL-6 was determined using the B9 mouse hybridoma cell line which depends on IL-6 for growth. CSF samples were heat-inactivated at 56°C for 30 min to remove inhibitory activity. 5000 cell/well were incubated with samples or standards in a 96 well microtitre plate for 4 days at 37°C in the presence of 5% CO2. Cell proliferation was evaluated colorimetrically by assaying for mitochondrial dehydrogenase using tetrazolium salt as substrate. The absorbance was recorded at 540 nm. The detection limit of the
assay was 3 pg/mL and the CV was less than 13% across the assay range up to 50 pg/mL.

Standardization of both immunoassay and bioassay was performed using the IL-6 international reference preparation (88/514).

**Gel permeation chromatography**
Following centrifugation, 200 µL of CSF samples were applied onto a Superose HR 200 gel filtration column (Pharmacia-LKB, Milton Keynes, UK). The FPLC column was attached to a Pharmacia-LKB chromatographic system and had been equilibrated with phosphate buffered saline containing 5 g/L BSA. Column fractions (1 mL) were collected and elution was monitored by recording absorbance at 280 nm. The column was calibrated using Sigma molecular weight markers. Fractions were stored at -70°C until analysed for both bioactive and immunoactive IL-6 using the above assays.

**Microbiological analysis of CSF**
An aliquot of each CSF sample was analysed by the Department of Medical Microbiology (Royal Liverpool University Hospital, Liverpool, UK) by microscopic examination, differential cell count and identification of the infective organism by cell culture and/or latex agglutination tests (Wellcome Diagnostics Limited, Dartford, UK).

**RESULTS**
Immunoactive and bioactive IL-6 was detectable in all CSF and serum samples analysed. Although the values obtained by both assays were significantly correlated ($r=0.72, P<0.001$; Fig. 1), most samples showed increased biological activity relative to immunoactivity, the bioactivity/immunoactivity ratio ranging from 0.2 to 24.3 (mean 4.6).

In order to investigate whether molecular heterogeneity of the cytokine was the cause of this discrepancy, CSF samples with B/I ratios of 1.0, 1.1, and 5.6 were applied onto a Superose gel permeation column. These studies were limited by the volume of CSF available and the extremes of the ratio could not be studied. A single peak corresponding to a molecular weight of 25 kDa was observed for all three CSF samples (Fig. 2 shows a typical elution profile). The bioactivity and immunoactivity were superimposable in these CSF samples. No other peaks were recorded by either assay, indicating the absence of bio- or immunoactive high molecular weight aggregates of the cytokine and/or low molecular weight fragments.
Dose response curves for selected CSF samples with low and high B/I ratios were parallel in both assays. Furthermore, bioactivity was completely abolished following addition of the polyclonal anti-IL-6 antiserum that was also used in the in-house immunoassay (data not shown). This demonstrates the specificity of the bioassay.

CSF samples were obtained from 246 patients, and analysed for total protein, glucose, lactate and for IL-6 concentrations. The number of assays depended upon availability of a suitable volume of CSF in an appropriate container. CSF samples were classified according to microbiological evidence of an infective agent (Table 1).

A total of 111 CSF samples showed no microbiological evidence of infection (neither bacteria nor their antigens detected in blood or CSF; and no raised neutrophil count) and were classified as normal, whereas 123 CSF samples showed microbiological evidence of bacterial infection with either Neisseria meningitidis (n = 121), or rarely Haemophilus influenzae (n = 2). Twelve CSF samples showed evidence of viral infection (enteroviruses, n = 7, measles, n = 2 and herpes simplex, n = 3).

Biochemical parameters measured were not normally distributed (Royston's development of the Shapiro-Francia W test). A non-parametric Wilcoxon rank sum test was therefore used to determine statistical significance and results are expressed as median and range (Table 1).

The total protein concentration in normal CSF (median 0.37 g/L) was significantly lower than in CSF samples in viral (median 0.67 g/L) or bacterial meningitis (median 2.75 g/L) (P < 0.01, and < 10^-7, respectively). Furthermore, total protein in CSF in bacterial infection was significantly higher than in viral infection (P < 10^-5; Fig. 3).

CSF lactate concentration in bacterial meningitis (median 18.25 mmol/L) was significantly higher than in normal CSF (median 1.75 mmol/L), and viral meningitis CSF (median 3.34 mmol/L) (P < 0.01) but there was no significant (P = 0.2) difference between viral and normal CSF lactate (Table 1).

Glucose concentration in viral meningitis CSF (median 3.2 mmol/L) was significantly (P < 0.01) lower than in normal CSF (median 3.6 mmol/L), and in bacterial meningitis CSF samples glucose concentration (median 0.5 mmol/L) was significantly lower than both viral meningitis and normal CSF (P < 0.001).

IL-6 concentrations in normal CSF (median 0.37 ng/mL) were significantly lower than in

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**Table 1.** Cerebrospinal fluid total protein, glucose, lactate and interleukin-6 (IL-6; immunoassay) concentrations and cell counts in different groups (median values, range in parentheses)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Total protein (g/L)</th>
<th>Glucose (mmol/L)</th>
<th>Lactate (mmol/L)</th>
<th>IL-6 (ng/mL)</th>
<th>Cell count (μL^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>0.37 (0.15-1.33)</td>
<td>3.6 (1.7-13.3)</td>
<td>1.75 (1.15-6.62)</td>
<td>0.37 (0.04-12.5)</td>
<td>&lt;1-262</td>
</tr>
<tr>
<td>[n = 93]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial meningitis</td>
<td>2.75 (0.1-10.95)</td>
<td>0.5 (0.1-4.9)</td>
<td>18.25 (8.95-35.75)</td>
<td>99.2 (0.04-707.9)</td>
<td>125-2000</td>
</tr>
<tr>
<td>[n = 109]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral meningitis</td>
<td>0.67 (0.24-1.22)</td>
<td>3.2 (0.3-5.4)</td>
<td>3.34 (0.06-6.32)</td>
<td>1.18 (0.097-12.5)</td>
<td>&lt;1-210</td>
</tr>
<tr>
<td>[n = 10]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = Number of samples analysed.
Cerebrospinal fluid IL-6 in meningitis

4·0 3·0 2·0 1·0 0·0

Serum IL-6 (ng/mL)

0·0 2·0 4·0 6·0 8·0 10·0 12·0 14·0

CSF IL-6 (ng/mL)

FIGURE 4. Correlation between cerebrospinal fluid (CSF) and plasma immunoreactive interleukin-6 (IL-6). Matched specimens from 13 patients with suspected bacterial meningitis and three with suspected viral meningitis.

Table 2. Test characteristics of cerebrospinal fluid interleukin-6 (at two different cut-off levels), total protein, and lactate in the diagnosis of bacterial meningitis

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (cut-off 3·4 ng/mL)</td>
<td>93·5</td>
<td>95·1</td>
<td>95·0</td>
<td>94·3</td>
</tr>
<tr>
<td>IL-6 (cut-off 12·5 ng/mL)</td>
<td>86·2</td>
<td>100</td>
<td>100</td>
<td>93·1</td>
</tr>
<tr>
<td>Total protein (ULN 0·45 g/L)</td>
<td>90·8</td>
<td>65·0</td>
<td>73·3</td>
<td>78·3</td>
</tr>
<tr>
<td>Lactate (ULN 3·5 mmol/L)</td>
<td>100</td>
<td>85·1</td>
<td>87·5</td>
<td>88·0</td>
</tr>
</tbody>
</table>

ULN = Upper limit of normal.

In this study CSF IL-6 was measured using two different assays, a bioassay using the B9 hybridoma cell line and an immunoassay using an in-house radioimmunoassay. Marked differences in biological (B) and immunological (I) activities for IL-6 were observed in CSF samples with B/I ratios ranging from 0·2 to 24·3.

Gel filtration experiments on selected CSF samples with different B/I ratios showed the presence of a single peak of about 25 kDa (expected for monomeric human IL-6). No other peaks of either bio- or immuno-activity were detectable, suggesting that the discrepancy in activities is not due to low molecular weight fragments or higher molecular weight aggregates of the cytokine. A further possibility is that a factor may be present which synergistically influences CSF IL-6 bioactivity.

Other cytokines may also stimulate B9 proliferation, for instance mouse but not human IL-4, thus, the presence of another factor and/or a cytokine stimulating B9 growth but with a similar molecular weight to IL-6 could explain the increased bioactivity.

As a result of this discrepancy in activities and because developments in immunoassays are more likely to result in rapid assays for the cytokine, only immunoactive CSF IL-6 data were used to investigate the diagnostic value of CSF IL-6 determination. CSF IL-6 concentrations in this study were significantly higher than those recently calculating the specificity, sensitivity, predictive value and efficiency of the tests (Table 2). The above test parameters were analysed at two proposed 'cut-off' points for CSF IL-6 concentrations 3·4 ng/mL and 12·5 ng/mL. IL-6 was found to have greater specificity and overall predictive value than the other biochemical tests, although lactate was found to have 100% sensitivity at a level of 3·5 mmol/L, the cut-off value suggested by other workers.

DISCUSSION

In this study CSF IL-6 was measured using two different assays, a bioassay using the B9 hybridoma cell line and an immunoassay using an in-house radioimmunoassay. Marked differences in biological (B) and immunological (I) activities for IL-6 were observed in CSF samples with B/I ratios ranging from 0·2 to 24·3.

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As a result of this discrepancy in activities and because developments in immunoassays are more likely to result in rapid assays for the cytokine, only immunoactive CSF IL-6 data were used to investigate the diagnostic value of CSF IL-6 determination. CSF IL-6 concentrations in this study were significantly higher than those recently
reported by Stearman and Southgate. This may reflect the different immunoassays used, or the size or type of population under study. Stearman and Southgate also found no significant differences in CSF IL-6 between bacterial and viral meningitis.

By contrast, in this study CSF samples from patients with bacterial meningitis showed significantly higher levels of IL-6 than those with either viral or with no detectable meningitis. However, there was some overlap, suggesting that although markedly elevated CSF IL-6 concentration is highly suggestive of bacterial meningitis, low CSF IL-6 does not exclude this diagnosis. It should also be noted that failure to identify organisms does not imply that these patients were normal—they were sufficiently ill to justify lumbar puncture and some clearly had protein/lactate results outside the normal range.

The diagnostic value of measuring CSF IL-6 was assessed by calculating the specificity, sensitivity, predictive value and efficiency of the test (Table 2) as described by Galen and Gambino. The above parameters were analysed at two 'cut-off' points for CSF IL-6, a low value (3-4 ng/mL) and a higher value (12-5 ng/mL). The specificity of the test at these two levels was 95·1% and 100%, respectively, whereas the sensitivity of the test was 93·5% and 86·2%, respectively. Prediction values at these cut-off levels were 95·0% and 100% and test efficiencies were 94·3% and 93·1%, respectively.

Using the lower cut-off value for CSF IL-6 allows the detection of more patients with bacterial meningitis. Although some would be classified as normal (6%) based on the IL-6 results, those patients will normally have high indices of suspicion and may undergo further examination. It is not clear why some CSF samples in bacterial meningitis showed low IL-6 levels in this study. Similar findings were reported by Rusconi et al. in admission samples in two patients. Serial CSF samples were not obtained in this study, however, Rusconi et al. showed that in four cases a higher IL-6 activity was detected in the CSF samples obtained 1–2 days after admission. This might be as a result of antibiotic therapy causing rapid bacterial lysis and associated with increased concentration of bacteria-free endotoxin and other cell wall components in CSF which induce the production of cytokines.

Bacterial meningitis is a medical emergency where intensive antibacterial therapy is essential. Therefore, a test with a high sensitivity is required to identify all patients with bacterial meningitis (few false negatives). Although treatment of false positives is undesirable due to potential side effects and toxicity, or colonization by opportunistic pathogens, delayed treatment of false negative results may be fatal and this outweighs risks associated with false positive results. Nevertheless, it is expected that the results of IL-6 assays would not be interpreted in isolation but could be part of a panel of investigations used to guide therapy.

It is clear that although CSF lactate measurement affords high sensitivity and specificity, it exhibits lower overall predictive value. However, conflicting data on CSF lactate have been reported. It should be noted that determining IL-6 in combination with CSF lactate improved the predictive value of the test to 93% or 97%, depending on the cut-off chosen for IL-6. It was unfortunate that, in the present study, a suitable specimen for lactate analysis was obtained in only a small proportion of all patients with bacterial meningitis.

IL-6 is known to stimulate immunoglobulin synthesis by activated B lymphocytes and this may account for the poor but significant correlation between CSF IL-6 and CSF total protein concentration. However, although CSF immunoglobulin levels were not determined in the present study, Houssiau et al. found no correlation between IL-6 levels and immunoglobulins.

Current routine ‘front-line’ investigations include the measurement of CSF total protein, glucose and lactate. There was a significant difference between all three groups for all the above analytes, except between normal and viral CSF lactate. In our study 16·3% of CSF samples were blood stained or haemolysed and thus unsuitable for protein analysis. Measurement of CSF total protein shows a low specificity when compared with that observed for CSF IL-6 or CSF lactate, and it has been suggested that total protein measurement is best used for monitoring treatment.

CSF glucose concentration is normally about 65% of that in plasma taken at the same time as the lumbar puncture. Low values are attributed to bacterial infection, to increased number of neutrophils, or to the presence of neoplasm. To interpret CSF glucose concentration, a plasma specimen must be obtained at the same time as the lumbar puncture. Furthermore, determination of CSF glucose requires the use of an appropriate sample tube, and in our study 17·0% of CSF samples were not received in a 'fluoride-oxalate'
tube rendering them unsuitable for glucose estimation. Since only 26% of our CSF samples were accompanied with a corresponding blood sample, it is difficult to comment on the diagnostic value of CSF glucose, although significantly lower CSF glucose values were observed in bacterial meningitis as compared with those from normal and viral meningitis.

Other markers have been investigated in meningitis, including CRP, ferritin and lysozyme. However, their interpretation usually depends upon measurement of blood concentrations and entry to the CSF, rather than production within the CSF itself.\textsuperscript{24}

IL-6 was detectable in all CSF samples examined in our study, in contrast to the paper by Houssiau \textit{et al.}\textsuperscript{13} who, using the 7TD1 cell line bioassay, were unable to detect any bioactive IL-6 in CSF from patients without infection and IL-6 was only elevated in about half of patients with viral and bacterial meningitis. This could be due to the fact that we examined acute samples, whereas, Houssiau \textit{et al.}\textsuperscript{13} examined CSF at different stages of the disease. Delayed sampling may result in false negative results and therefore only early samples should be measured.

CSF IL-6 is mainly produced intrathecally, since there was no correlation between CSF and serum IL-6 concentrations. This finding is in agreement with other reports.\textsuperscript{13,16}

Analysis of the kinetics of IL-6 in CSF and the relationship between the various inflammatory indices in man is complicated by the difficulty in obtaining a reliable admission history of the study patients, the different therapeutic approaches after hospital admission (in the case of delayed samples) and the lack of repeated CSF samples after initiation of therapy.

Although elevated CSF IL-6 levels are strongly suggestive of bacterial meningitis, low levels do not rule out the infection. Normals and those with viral meningitis show low levels of IL-6. For the determination of CSF IL-6 to be of diagnostic value, a rapid immunoassay is essential. A number of ELISAs are commercially available, but they are expensive. We have recently developed a rapid (<80 min) in-house ELISA, and preliminary results correlate with the radioimmunoassay (unpublished data). It is likely that measurement of CSF IL-6 will prove useful in the differential diagnosis of bacterial meningitis. Furthermore, there are advantages in that only a CSF specimen is required with no need for a corresponding blood specimen and the test is not affected by haemolysis.

IL-6 is fairly stable and no special precautions are required for specimen collection, transport and storage. However, sample should be centrifuged early, since prolonged contact with cells may result in false elevated IL-6 results due to \textit{in vitro} production by leucocytes.

CONCLUSION

Our data indicates that IL-6 measurement in CSF may assist the early diagnosis and discrimination of infective meningitis.

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