Transcranial cytokine gradients in patients requiring intensive care after acute brain injury

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Summary
After acute brain injury there may be increased intracranial production of cytokines, with activation of inflammatory cascades. We have sought to determine if a transcranial cytokine gradient was demonstrable in paired sera of 32 patients requiring intensive care after acute brain injury. The difference between concentrations of IL-1β, IL-6, IL-8 and TNFα in jugular venous and arterial serum was measured on admission, and at 24, 48 and 96 h after the primary injury. There were no differences in IL-1β, IL-8 or TNFα, but median gradients of 6.7 and 11.5 pg ml⁻¹ for IL-6 were demonstrated in the traumatic brain injury (n=22) and subarachnoid haemorrhage (n=10) groups, respectively (normal values in serum <4.7 pg ml⁻¹; P<0.001 both groups). This suggests that there is significant production of IL-6 by intracranial cells after acute brain injury. Therapy directed towards combatting the negative effects of IL-6 may potentially benefit patients who have sustained an acute brain injury. (Br. J. Anaesth. 1997; 78: 520–523).

Key words

The cytokines are a variety of polypeptide molecules which function as mediators within the communication network of the immune system.¹ They have been implicated in the pathophysiology of many disease processes, including the systemic inflammatory response syndrome (SIRS),² acute respiratory distress syndrome (ARDS)³ and multiple trauma.⁴ Many of the biological effects of the cytokines are clinically evident after acute brain injury; these include neutrophilia, pyrexia and alteration of endothelial permeability,⁵ which may result in cerebral oedema,⁶ as a result of disruption of blood–brain barrier function.

Increased concentrations of serum tumour necrosis factor-α (TNFα),⁷ interleukin-6 (IL-6)⁸ and intraventricular fluid IL-6 and IL-1β⁹ have been demonstrated after traumatic brain injury, and experimental work has demonstrated that glial cells are able to manufacture cytokines.¹⁰¹¹ After acute brain injury it has been suggested that increased intracranial production of pro-inflammatory cytokines, with consequent induction of auto-destructive inflammatory cascades, results in secondary injury to the brain, causing altered brain metabolism and cell death.¹² With the increased use of intra-parenchymal solid state methods for monitoring intracranial pressure, access to cerebrospinal fluid for analysis is often not possible, and where it is, sampling from catheters may result in ventriculitis.¹³ Insertion of a fiberoptic catheter into the jugular bulb to monitor jugular venous haemoglobin oxygen saturation is now standard practice within our intensive care unit (ICU) in acute brain injured patients, giving ready access to blood draining from the brain.¹⁴ We investigated the hypothesis that systemic concentrations of the cytokines IL-1β, IL-6, IL-8 and TNFα are increased after acute brain injury, and that increased intracranial production of these cytokines results in a demonstrable transcranial cytokine gradient, with jugular venous concentrations higher than arterial concentrations.

Patients and methods
After obtaining local Ethics Committee approval, we studied 32 patients who had sustained an acute brain injury (traumatic brain injury (TBI) or spontaneous subarachnoid haemorrhage (SAH)) requiring intensive care. Patient data consisting of sex, age and Glasgow coma score (GCS) after non-surgical resuscitation were recorded on admission to the ICU (see table 1). One patient whose initial GCS was recorded as 15 was anaesthetized at the scene soon after injury because of flail chest. Computerized tomography (CT) subsequently demonstrated severe traumatic brain injury.

A dual-lumen Edslab 4-French gauge oximetry catheter (Baxter Healthcare Corporation, Irvine, CA, USA) was inserted into the jugular bulb on the dominant side of the cerebral venous drainage, as described previously.¹⁵¹⁶ Satisfactory positioning was ensured by lateral x-ray of the cervical spine (catheter tip seen cephalad to the upper border of the C2 vertebral body), and the correct catheter distance marking (e.g. 15 cm) showing at the valve of the sheath was written in the notes for future
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Reference. An intra-arterial catheter was inserted (if not already in situ), usually in the radial artery, as is our standard practice. Paired arterial and jugular venous blood samples were obtained at designated times after brain injury: within 12 h (median 8 h 30 min, range 2–14 h), and at 24, 48 and 96 h. Time of brain injury in the SAH group was noted as the time of sudden deterioration in GCS by 3 points or more. Patients who did not exhibit sudden neurological deterioration were not included. The diagnostic criterion for SAH was the presence of subarachnoid blood on the CT scan. Satisfactory positioning of the jugular bulb catheter was ensured before each sample was obtained by checking that the correct distance marker of the catheter was showing at the sheath valve. Samples were allowed to clot at room temperature, spun down in a centrifuge at 4000 rpm for 10 min and the supernatant removed and frozen immediately at −25 °C. Analysis of serum for IL-1β, IL-6, IL-8 and TNFα was performed by enzyme-linked immunosorbent assay (Quantikine Human Cytokine Assays, R&D Systems) according to the manufacturer’s instructions. Inter- and intra-assay coefficients of variation for each assay are shown in table 2. All analyses were carried out by a single operator using the same equipment and procedures throughout. Repeat freeze-thaw cycles for serum samples were avoided by separating the serum from each patient into four tubes before freezing.

A total of 138 samples (69 pairs) were analysed in duplicate for each cytokine (with an additional 80 samples for IL-6) and results averaged to give the final concentration. Samples which gave a result higher than the highest standard supplied with the kit were diluted and the assay repeated. Sera from eight healthy volunteers were analysed as controls. Standard curves and cytokine concentrations were calculated on a personal computer interfaced to a micro-plate reader using dedicated software (Bio-Rad Laboratories). Statistical analysis was by Wilcoxon rank sum and signed rank tests using Splus for Windows 3.2.

Results

In 119 of the 138 samples assayed for IL-1β (86%), concentrations were undetectable (<3.9 pg ml⁻¹). The highest concentration in the remaining 19 (14%) was 7.0 pg ml⁻¹. For TNFα, in 122 samples (88%) concentrations were undetectable (<15.6 pg ml⁻¹). The highest concentration in the remaining 16 (12%) was 31 pg ml⁻¹. IL-8 was detected in 72 samples (52%) (lower limit of detection 94 pg ml⁻¹), but only 10 (7%) showed concentrations >300 pg ml⁻¹. There was no difference between jugular venous and arterial concentrations of IL-1β, IL-6 or TNFα in those patients where arterial and jugular venous blood samples were obtained at designated times after brain injury: within 12 h (median 8 h 30 min, range 2–14 h), and at 24, 48 and 96 h. Time of brain injury in the SAH group was noted as the time of sudden deterioration in GCS by 3 points or more. Patients who did not exhibit sudden neurological deterioration were not included. The diagnostic criterion for SAH was the presence of subarachnoid blood on the CT scan. Satisfactory positioning of the jugular bulb catheter was ensured before each sample was obtained by checking that the correct distance marker of the catheter was showing at the sheath valve. Samples were allowed to clot at room temperature, spun down in a centrifuge at 4000 rpm for 10 min and the supernatant removed and frozen immediately at −25 °C. Analysis of serum for IL-1β, IL-6, IL-8 and TNFα was performed by enzyme-linked immunosorbent assay (Quantikine Human Cytokine Assays, R&D Systems) according to the manufacturer’s instructions. Inter- and intra-assay coefficients of variation for each assay are shown in table 2. All analyses were carried out by a single operator using the same equipment and procedures throughout. Repeat freeze-thaw cycles for serum samples were avoided by separating the serum from each patient into four tubes before freezing.

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![Figure 1](https://example.com/figure1.png)

**Figure 1** Differences between jugular venous and arterial concentrations of IL-6 in traumatic brain injury (TBI, n = 22) and spontaneous subarachnoid haemorrhage (SAH, n = 10), over a 4-day period after injury (A = admission). Data may be incomplete because of patient death, discharge from ICU or values lying outside ELISA quantification values. For the TBI group, at each time, n = 20, 21, 19 and 11, respectively; for the SAH group, n = 10, 10, 8 and 6, respectively.

![Table 1](https://example.com/table1.png)

**Table 1** Characteristics of patient population (median (range)). The characteristics of the subset, including only the first 20 patients, are shown in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>TBI group (n = 22 (12))</th>
<th>SAH group (n = 10 (8))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>18/4 (9/3)</td>
<td>6/4 (4/4)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>34 (35)</td>
<td>52 (51.5)</td>
</tr>
<tr>
<td>GCS</td>
<td>7 (7)</td>
<td>7 (7)</td>
</tr>
</tbody>
</table>

![Table 2](https://example.com/table2.png)

**Table 2** Performance characteristics of each assay. To assess intra-assay precision, three samples of known concentration were assayed 20 times on one plate. To assess inter-assay precision, three samples of known concentration were assayed in 20 separate assays.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intra-assay coefficient of variation (%)</th>
<th>Inter-assay coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
<tr>
<td>IL-1β</td>
<td>3.4 4.4 2.8</td>
<td>8.4 4.2 4.1</td>
</tr>
<tr>
<td>IL-6</td>
<td>4.3 1.7 2.1</td>
<td>6.3 3.3 7.2</td>
</tr>
<tr>
<td>IL-8</td>
<td>3.9 2.4 3.3</td>
<td>12.2 9.1 7.3</td>
</tr>
<tr>
<td>TNFα</td>
<td>5.2 4.2 4.6</td>
<td>7.4 4.6 5.4</td>
</tr>
</tbody>
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were allowed to clot at room temperature, spun down in a centrifuge at 4000 rpm for 10 min and the supernatant removed and frozen immediately at −25 °C. Analysis of serum for IL-1β, IL-6, IL-8 and TNFα was performed by enzyme-linked immunosorbent assay (Quantikine Human Cytokine Assays, R&D Systems) according to the manufacturer’s instructions. Inter- and intra-assay coefficients of variation for each assay are shown in table 2. All analyses were carried out by a single operator using the same equipment and procedures throughout. Repeat freeze-thaw cycles for serum samples were avoided by separating the serum from each patient into four tubes before freezing.

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with detectable concentrations. Control samples all showed undetectable concentrations.

IL-6 was detected in all 218 samples. In the controls, concentrations were below detectable limits (<3.1 pg ml\(^{-1}\)) in four samples. The highest concentration in the remaining four control samples was 4.7 pg ml\(^{-1}\). In four pairs of patient samples (3.7%), both showed concentrations >1500 pg ml\(^{-1}\) (the upper limit of quantification) and were excluded from statistical analysis. In 82 (78.1%) of the remaining 105 pairs, jugular venous concentration was higher than arterial. In the TBI group, median jugular venous concentration was 74.8 (range 19.9–539.8) pg ml\(^{-1}\), median arterial concentration was 63.5 (11.4–518.4) pg ml\(^{-1}\) and the median difference was 6.7 (−40.8 to 244.4) pg ml\(^{-1}\) (P<0.001). In the SAH group, median jugular venous concentration was 64.6 (19.1–455.4) pg ml\(^{-1}\), median arterial concentration was 54.1 (12.2 to 407.4) pg ml\(^{-1}\) and the median difference was 11.3 (−32 to 135.2) pg ml\(^{-1}\) (P<0.001).

Figure 1 shows the individual patient profiles for transcranial IL-6 gradient for each group. In the TBI group, median gradients at each time point were 19.2 (P<0.001), 10.5 (P=0.014), 6.3 (P=0.018) and 1.6 pg ml\(^{-1}\) (P=0.147), respectively. In the SAH group, median gradients were 26.8 (P=0.002), 20.1 (P=0.027), 5.6 (P=0.383) and 7.8 pg ml\(^{-1}\) (P=0.094), respectively. Comparing the TBI and SAH groups, there was a significant difference in age between the groups (P=0.028), but there was no difference in GCS (P=0.979). There was no overall difference in IL-6 gradients across the brain between the TBI and SAH groups (P=0.350); there were no differences between groups at any of the four sampling times (P=0.559, 0.627, 0.832, 0.366, respectively).

Discussion

We were unable to derive any useful information from measurement of either arterial or jugular venous serum concentrations of IL-1β, IL-8 or TNFα at these times after acute brain injury. It is generally believed that IL-1β and TNFα are released in the early post-injury phase, resulting in increased synthesis of IL-6, in addition to other mediators. IL-6 may act to decrease production of IL-1β and TNFα via a negative feedback mechanism. It may be that we missed an early peak of production of IL-1β and TNFα before the patients reached the ICU. The relatively low lower limit of detection for IL-8 (94 pg ml\(^{-1}\)) in this particular assay may have masked any significant transcranial gradient.

Results for IL-6 show increased jugular venous concentrations of this cytokine relative to arterial concentrations, particularly within 48 h of brain injury. Transcranial gradients in the TBI and SAH groups were similar, despite different aetiologies of primary injury, suggesting that the mechanisms of the inflammatory process may be similar. The median transcranial gradient on admission in each group was 5–6 times higher than the highest systemic concentration measured in the controls. This suggests significant intracranial production of IL-6. The source of this increased production is likely to be glial cells, and there is experimental evidence to suggest that both astrocytes and microglial cells may be involved. In addition, systemic concentrations of IL-6 were increased greatly. This may be caused solely by intracranial production, but is much more likely to be a result of release of IL-6 from extracranial sources, that is a mild SIRS. Variation in systemic concentrations of IL-6, jugular venous–arterial differences, and different patterns of concentration gradients seen over time may be related to the severity of intracranial injury, type of injury, presence of extracranial injuries or secondary insults to the brain while in the ICU.

This pattern of prolonged elevation of IL-6 is seen in SIRS and ARDS, and serum concentrations of IL-6 have been shown to be related to outcome in these conditions, both in terms of maximum concentrations detected and persistence of IL-6 in serum over time. There has been little work relating outcome in acute brain injury to IL-6 concentrations in serum. We are presently collecting outcome data for our group of patients.

A study which compared post-mortem appearances of the brains of non-survivors of traumatic brain injury in Glasgow over two periods (1968–1972 and 1981–1982) showed that the incidence of ischaemic damage had not changed, despite improved intensive care in this group of patients. Analysis of our own secondary insult database of physiological variables for acute brain injured patients in the ICU revealed a significant reduction in hypotensive and hypoxic insults (i.e. periods where mean arterial pressure and arterial haemoglobin oxygen saturation decreased below acceptable limits) to the brain over a 7-yr period, but this has not been paralleled by a similar reduction in mortality. We can speculate that in inflammatory disease states the mediators produced may prevent cells from using oxygen supplied to tissues, that is there exists a state of histotoxic hypoxia. This may explain why therapeutic efforts directed at increasing oxygen delivery to tissues have not resulted in improved outcome in SIRS. If this hypothesis is correct then a different therapeutic approach is necessary.

Future management of acute brain injured patients may include pharmacological modification of the inflammatory processes involved in the pathophysiology of the condition, using antibodies or receptor antagonists, preventing further cell death and improving outcome.

References

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