Short report

Blood-stained cerebrospinal fluid: traumatic puncture or haemorrhage?

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Summary Computed tomography fails to ascertain, or exclude, the presence of intracranial haemorrhage in a considerable number of cases, especially in subarachnoid haemorrhage and haemorrhagic infarcts. A number of other methods, including cerebrospinal fluid spectrophotometry and cytology have, therefore, been tested to define their diagnostic efficacy in 25 cases of confirmed intracranial haemorrhage and in 25 instances of blood-stained cerebrospinal fluid due to traumatic puncture. The combination of spectrophotometry and cytology proved to have a high diagnostic reliability. On the basis of these results a routine scheme of investigation is proposed.

It is of major importance to ascertain whether blood-stained cerebrospinal fluid (CSF) is the result of intracranial or intraspinal haemorrhage, or due to traumatic puncture, particularly when anti-coagulant therapy is to be considered. In order to decide whether blood-stained CSF is an indication of a pathological process, Barrows et al.1 recommended the spectrophotometric method. This, along with a number of other widely used methods, has been lucidly evaluated by Tourtelotte et al.2 Study of the cytology of CSF, with detection of erythrophages, can now be added to this list. Although computed tomography is a valuable tool when haemorrhage is to be excluded, it is well known that in haemorrhagic infarcts isodensity may occur, while in traumatic as well as non-traumatic subarachnoid haemorrhages false negative results may also occur.3,4 The necessity for a simple and effective method to exclude intracranial haemorrhage prompted us to study once again the efficacy of a number of methods and combination of methods.

Materials and methods

Every blood-stained CSF sample produced was investigated according to standard procedures, until we had collected 25 cases in which intracranial haemorrhage could be confirmed (Group 1), and another 25 cases in which haemorrhage was considered highly unlikely (Group 2). In the first group the presence of intracranial haemorrhage was proven by computed tomography, either by demonstrating locally increased attenuation or by confirming the presence of severe cerebral contusion. Diagnoses of the patients in this group were: contusion (15 patients) intracerebral haemorrhage (8 patients), subarachnoid haemorrhage (2 patients). In Group 2 haemorrhage was excluded on clinical grounds and also by computed tomography in the patients with intracranial disorders. Diagnoses in this second group were: epilepsy (6), dementia (4), extrapyramidal disorders (4), cerebral tumour (2). In the other patients the diagnosis was polyneuropathy or there were no neurological abnormalities. The presence of haemorrhage in this group is, of course, not completely excluded. Serum bilirubin was normal in all patients. Lumbar punctures were performed in the third and fourth lumbar interspaces. The operator stated whether he thought the puncture to be traumatic. CSF was collected in at least three consecutive tubes, and a red blood cell (RBC) count was performed in each tube. To test whether the RBC counts...
decreased in three consecutive tubes, Page's test (trend analysis of repeated series of observations9) was used for each group separately. To quantify the (linear) decrease per person, the Pearson correlation coefficient between the three RBC counts and the corresponding sequence number of tubes, was used. Samples for cytological studies were taken from the tube with the lowest number of RBCs.

All tubes were centrifuged immediately. This implies that no more than 0.5–3% haemolysis of the RBCs occurred.2,6 The combined CSF supernatants of the three tubes were inspected and described as colourless or yellow. Then spectrophotometry was performed on the supernatant (Varion 634 double-beam spectrophotometer). Optical density was screened at wavelength 415 nm. Extinctions below 0.023 were considered to indicate the absence of blood pigment.7 If the extinction exceeded 0.023 an absorption curve was plotted by measuring the optical density on the wavelength range 400–600 nm.8 Extinctions exceeding 0.023 with a maximum at 415 nm were considered to indicate the presence of oxyhaemoglobin. If the absorption curve revealed a visual shoulder at 455 nm, this was regarded as proving the presence of bilirubin. If only oxyhaemoglobin was found, the puncture was considered to be traumatic, since the interval between the onset of the intracranial haemorrhage and puncture was more than two to three hours in all patients.1,8 Sediments for cytological examination were prepared from 1 ml of CSF according to Sayk9 and stained with May-Grünwald-Giemsa.

Results and discussion

Collection of CSF in three tubes and the counting of the RBCs in each subsequent tube, although time consuming, is simple, and does not require elaborate equipment. A decrease was found in the sequential RBC count of the subsequent samples of Group 2 (p<0.00012). No such decrease could be demonstrated in Group 1 (p<0.25).

To find out whether the presence of a regular decrease in RBC’s would be sufficient to allocate the individual case to Group 1 or 2, the Pearson correlation coefficients were compared for the two groups. Although there is a significant difference between the two groups, no proper distinction could be made for the individual case (see table). Changing the allocation criterion used, for example 1.00 ≤ r ≤ 0.80 did not improve discrimination. In this respect one should also bear in mind that the results in this study are based on groups with the same number of individuals. In clinical practice, however, the frequency distribution of haemorrhages versus artificial puncture is unknown and differs with the skill of the operator. This unfortunately implies that the method does not provide the clinician with a tool to predict whether a particular patient is likely to belong to Group 1 or 2. The results of the inspection of the supernatant, spectrophotometry and CSF cytology are presented in the figure. Xanthochromia appears to have a probability score of 73% (95% confidence interval 54–88%) for a particular patient to belong to Group 1 (figure, A). A colourless supernatant allocates the patient with a probability score of 85% (95% confidence interval 62–97%) to Group 2. The fact that three false negative instances occurred in the haemorrhage group implies that the method is not capable of excluding haemorrhages, whereas a yellow supernatant does not allow any conclusions. In this series screening of the supernatant by spectrophotometry at 415 nm appears to allow the exclusion of haemorrhage, but shows the same disadvantages as the mere inspection of the supernatant in the traumatic puncture group (figure, B).

To get rid of the false positive cases in this group, an absorption curve has to be plotted to measure whether the extinction is due to oxyhaemoglobin, to bilirubin, or to a combination of these. Only one of the eight false positive instances presented in the figure, A and B, appeared to be positive for bilirubin (figure, C). In fact, only one of the 25 cases of artificial punctures showed traces of bilirubin. Although the absence of bilirubin thus appears to indicate that the puncture was traumatic, the small number of investigated patients does not allow a definite conclusion. In the haemorrhage group the absorption curve showed, besides oxyhaemoglobin, the presence of bilirubin with a trivial shoulder at 455 nm in two cases and a characteristic peak in all the others (figure, C). If bilirubin is present, this is highly indicative of real haemorrhage, but there is still a 4% chance (95% confidence interval 0.1–20.3%) that the puncture was traumatic, that is, if haemorrhage and traumatic puncture occur with the same frequency. If cytology demonstrates the presence of

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Table Distribution of predicted intracranial haemorrhage and traumatic punctures based on the decreased RBC content in subsequent CSF samples

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<tr>
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<th>Intracranial haemorrhage</th>
<th>Traumatic puncture</th>
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<tbody>
<tr>
<td>Intracranial haemorrhages (Group 1, n=25)</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Traumatic punctures (Group 2, n=25)</td>
<td>11</td>
<td>14</td>
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*Allocation criterion: all RBC counts with a coefficient of correlation −1.00 ≤ r ≤ 0.90 were attributed to artificial bleeding.
erythrocytes, this evidently proves the presence of haemorrhage (figure, D); negative cytology, however, does not exclude haemorrhage.

With respect to the opinion of the operator, in Group 1 the puncture was falsely thought to be traumatic in two instances. In all cases of Group 2 the impression appeared to be correct. One might feel, therefore, that all the elaborate techniques can hardly compete with the opinion of the operator. However, since a number of factors that influence the opinion are derived from clinical data of the punctured patients, these results give a false impression. The clinical data in the two groups studied here are by definition rather explicitly pointing to haemorrhage or artificial puncture, since the groups were selected that way. Furthermore, the impression of the operator appeared not to be registered in seven cases from both groups. It may well be that the operator was unable to decide in these instances. If all the methods studied here are available, the following scheme seems advisable. First, spectrophotometric screening of the supernatant at a wave-length of 415 nm should be carried out. If the extinction is below 0.023, haemorrhage can almost certainly be excluded. If the extinction is above 0.023, an absorption curve should be plotted; if no bilirubin can thus be detected, haemorrhage can again be excluded; if bilirubin is detected, haemorrhage is very likely, but due to one case from the artificial puncture group with a trace of bilirubin, no absolutely definite conclusion can be drawn. To

Figure (A) appearance of the supernatant, (B) extinction at 415 nm, (C) spectrophotometric results. (In one case the supernatant was turbid and no measurements could be made. In one patient methaemoglobin was present instead of oxyhaemoglobin, accounting for the shorter left side of the first column.) (D) cytology of the CSF.
exclude the slight possibility of traumatic puncture, CSF cytology should be performed. If erythropages are present, the diagnosis of haemorrhage can be made with certainty. If, however, cytology is negative, haemorrhage is not excluded.

When blood-stained CSF is obtained, it appears advisable, even in the era of CT scanning, to follow the lines of investigation described above to differentiate between haemorrhage and traumatic puncture.

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References