Differentiation of Early Subarachnoid Hemorrhage from Traumatic Lumbar Puncture

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SUMMARY The problem of early differentiation of "traumatic tap" from subarachnoid hemorrhage (SAH) was studied in the rabbit by determining the changes in percentage of hemolysis and in lactate concentrations in CSF within the first twenty-four hours following induced SAH. The 0.3 to 7% hemolysis which occurred was relatively independent both of the time following SAH and of the number of red blood cells (rbc) in the cerebrospinal fluid (CSF).

HOW TO DISTINGUISH true subarachnoid hemorrhage (SAH) from accidental blood contamination of the cerebrospinal fluid (CSF) at lumbar puncture, or so-called "traumatic tap," has been investigated for many years, and a number of clinically useful methods have been proposed. Unfortunately, many instances are encountered in which existing methods fail; these tend to be found in patients whose neurologic illness has been present only a few hours and in whom the need for a prompt diagnosis is greatest. Since the crucial difference between true SAH and "traumatic tap" is the length of time the blood has been present in the CSF, we considered several time-dependent processes for investigation, and finally selected two for study.

Tourtellotte et al. stated that 0.5 to 3% hemolysis occurs soon after red blood cells (rbc's) are introduced into the CSF and that essentially no further hemolysis occurs for at least six hours if the sample is stored at room temperature or at 4°C. Others found previously that incubation at 37°C resulted in progressive hemolysis which was easily detectable at one-two hours. This suggests that progressive hemolysis also occurs with time after SAH, which might be considered to be similar to 37°C incubation, but a maximum of 3% hemolysis might be found following a "traumatic tap" providing the sample is kept between 4°C and room temperature for less than 6 hours prior to measurement. We elected to test this hypothesis as one possible approach to the problem of differentiating recent SAH from traumatic lumbar puncture.

A second hypothesis which seemed clinically feasible is based upon the fact that incubated red blood cells produce lactate as a result of an aerobic glycolytic pathway. If other sources of lactate can be excluded, lactate concentration in hemorrhagic CSF should increase as a function of time following the onset of a subarachnoid hemorrhage, whereas the lactate concentration in the spinal fluid obtained from a "traumatic tap" should be identical with that of uncontaminated fluid if determined immediately.

Preliminary work involving in vitro incubation of autologous rbc's in CSF of patients with non-hemorrhagic neurologic disease revealed that less than 3% hemolysis occurred between 0 and 24 hours. Obvious differences exist between in vitro and in vivo incubation (e.g. the conversion of oxyhemoglobin to bilirubin, significant sequestration of rbc's in the subarachnoid space, and the possibility of introduction of lactate from other sources besides blood cell metabolism). So we elected to study both percent hemolysis and CSF lactate concentration as a function of time following experimental SAH in rabbits.

Methods

Twenty-two adult Kingswheel and New Zealand white rabbits were anesthetized with an intramuscular injection of 18 mg/kg of chlorpromazine and 55 mg/kg of ketamine, supplemented as needed with diethyl ether. In seven animals percutaneous cisternal puncture was performed using a 1½ inch, 22 gauge needle attached to a 1 ml syringe, and ½ ml of autologous cardiac blood was injected slowly into the cisterna magna. The rabbit was then allowed to awaken. Four hours later, the animal was re-anesthetized with diethyl ether, another cisternal puncture was performed and ½ ml of CSF was removed for study. A simultaneous arterial blood sample was obtained. A group of four animals was similarly treated, except that the second cisternal puncture was performed one hour after the introduction of autologous cardiac blood; since these animals had not yet regained normal alertness by that time, it was believed that persisting alterations in blood gas values would invalidate lactate and pyruvate data. Therefore, the CSF samples from these animals were used only to calculate percentage of hemolysis. Control data were obtained from eleven rabbits treated in the same way, except that no cisternal puncture was performed during the initial anesthetic procedure; the animal was allowed to awaken, and after four hours was re-anesthetized with diethyl ether and a ½ ml sample of CSF was obtained via cisternal puncture. A simultaneous arterial blood sample was also obtained as before. Seven animals from this group then had ½ ml of autologous cardiac blood injected into the cisterna magna immediately following removal of the control CSF sample. The animal was then allowed to awaken and was re-anesthetized 24 hours later and the final cisternal CSF sample obtained. Arterial blood samples were also utilized for gas determinations and for lactate and pyruvate levels. CSF was analyzed for hemoglobin, bilirubin, lactate and pyruvate as described below.

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All CSF specimens were centrifuged at 3000 rpm (1,800 × g) for ten minutes within fifteen minutes of collection. It was found that centrifugation for less than eight to ten minutes or at a lower speed usually resulted in incomplete sedimentation of rbc's in both human and rabbit CSF. Immediately after centrifugation the supernatant was removed carefully with a transfer pipette and placed in a stopped test tube. A volume of distilled water equal to the volume of supernatant removed was added to the sediments cells, and the tube frozen to hemolyze the sample. A portion of the supernatant was pipetted at the proportion of 1 ml to 3 ml of cold 5% (w/v) metaphosphoric acid to precipitate proteins. After mixing and centrifugation, the resulting supernatant was separated and frozen for subsequent lactate and pyruvate assays. The remainder of the original supernatant was used for calculation of percentage of hemolysis.

**Percentage of Hemolysis**

Optical density of the spinal fluid supernatant was determined at 412 and 480 μM using a Beckman D.U. Spectrophotometer. Microcuvettes having a width of 3 mm and a 1 cm light path were employed. The total hemoglobin concentration in the supernatant was then calculated using the following expression:

\[ C_{HB} = 8.7 \times (O.D._{412}) - 7.8 \times (O.D._{480}) - 0.1 \]

Where:

- \( C_{HB} \) = Hemoglobin concentration in μM
- O.D._{412} and O.D._{480} refer to the optical densities at 412 and 480 μm respectively

The tube containing the hemolyzed sediment was then thawed and after vortex mixing, the optical density of the hemolysate was determined in the same manner as that of the supernatant. It was often necessary to dilute the hemolysate (or both samples) if the optical density was too high; 0.067M phosphate buffer, pH 6.8, was used for this purpose.

The percentage of hemolysis may be calculated from these data using the expression:

\[ \text{Percentage of hemolysis} = \frac{C_{HB} \text{ (supernatant)}}{C_{HB} \text{ (hemolysate)}} \times 100 \]

Total rbc count may be calculated as follows:

\[ \text{rbc/mm}^3 = \frac{C_{HB} \text{ (hemolysate)}}{810} \]

This is derived from the following data:

1 μM hemoglobin = 1.7 mg% = 17,000 μg/mm³

and

1 rabbit rbc contains 21 μg of hemoglobin\(^*\)

so

\[ \text{rbc/mm}^3/μM \text{ Hb} = \frac{17000}{21} = 810 \]

The bilirubin concentration may be calculated by use of the following expression:\(^*\)

\[ C_B = 29 \times (O.D._{440}) - 1.8 \times (O.D._{450}) - .2 \]

Where:

- \( C_B \) = bilirubin concentration in μM

**CSF Lactate and Pyruvate**

CSF lactate was determined by the lactate dehydrogenase method.\(^1^1\),\(^1^3\) A glycine-hydrazine buffer was employed, and the change in O.D. was read at 340 μm.

CSF pyruvate was assayed by a somewhat similar procedure developed by Sigma Chemical Company.\(^1^8\) A Trizma\(^*\) buffer was used, and the change in O.D. at 340 μm measured.

**Results**

(A) Eighteen rabbits were studied at intervals varying from one to 24 hours following subarachnoid hemorrhage, and percentage of hemolysis values between 0.3 and 7% were obtained. Though these values were independent both of the time after subarachnoidal hemorrhage and of the magnitude of rbc count in the CSF, higher values tended to occur somewhat more frequently at the longer time intervals (table 1). Since generally lower rbc counts were also observed at these longer time intervals, despite the original introduction of equal volumes of autologous blood, the higher values probably reflect the sequestration of blood in the subarachnoid space\(^*\) rather than a greater quantity of lysed rbc's. Rbc counts varied from 14,200 to 1,100,000 rbc/mm³ for all experiments. CSF bilirubin was calculated for each sample, and in no case was the bilirubin concentration greater than the hemoglobin concentration\(^*\) (table 1).

(B) CSF lactate and pyruvate concentrations were determined in 11 control rabbits which had been anesthetized, allowed to awaken, then re-anesthetized at four hours in order to eliminate the question of a residual effect of alterations of blood gases due to the first anesthetic procedure\(^*\) when compared with those values obtained at four hours after subarachnoidal hemorrhage (table 2). Average CSF lactate concentration (control) was 1.99 mEq/L ± 0.12 S.E.M. Lactate and pyruvate concentrations from this control group were also utilized to calculate the lactate/pyruvate ratio\(^*\) which provides a means of detecting significant excess lactate production as a result of hypocapnea or other factors leading to cerebral ischemia, hypoxia, or reduction in the phosphate potential.\(^1^4\) The average lactate/pyruvate (L/P) ratio for this group was 11.0 (table 2).

Seven rabbits were studied four hours after subarachnoidal hemorrhage (table 3); the average value for CSF lactate in this group had increased to 3.16 mEq/± 0.24 S.E.M. and the L/P ratio had increased slightly to 12.7. The increase in lactate concentration is significant (p < .001) (Student’s t test), while the increase in L/P ratio is not (p < 0.2). Seven rabbits were studied 24 hours after subarachnoidal hemorrhage, when the average CSF lactate had dropped to 2.46 mEq/L ± 0.13 S.E.M., with L/P ratio of 11.0 (table 4). The decrease in lactate concentration at 24 hours may reflect the clearance or isolation of rbc's from the subarachnoid space, since average CSF rbc counts decreased from 846,000 in one hour samples to 467,000 at four hours and 170,000 by 24 hours despite the introduction of equal volumes of blood at zero time. As noted by others\(^3\),\(^1^4\),\(^1^7\) there was no correlation with CSF data providing the Pco₂ and Po₂ were within reasonable limits (tables 2, 3).
general agreement with the in vitro findings of Tourtellotte et al. However, provide quantitative data. Matthews and Frommeyer also investigated the hemolysis of both homologous and heterologous rbc's in CSF, and reported a gradual decrease in number of rbc's during the first 12 hours of incubation at 37.5°C, with increasing xanthochromia "visible to the naked eye" by four hours with rbc counts of 1,000 rbc/mm³ or more. Many other investigators refer to the appearance of significant xanthochromia in subarachnoid hemorrhage within four hours or less of the event. It is possible that, in addition to oxyhemoglobin, this may reflect serum contamination, increased CSF protein, or early conversion of hemoglobin to bilirubin.

It is clear from the data presented that progressive hemolysis does not occur in CSF under conditions of simulated subarachnoid hemorrhage, at least within the time interval prior to the expected appearance of bilirubin. Early diagnosis of subarachnoid hemorrhage therefore cannot be made by the degree of hemolysis in the CSF.

Our data demonstrate a significant increase in CSF lactate
concentration at four hours after experimental subarachnoid hemorrhage, followed by a decrease of approximately half that amount by 24 hours. An attempt was made to identify other potential sources of increased CSF lactate such as hypocapnea or hypoxia.\(^{17}\) by determining the cardiac blood pH, PO\(_2\), PCO\(_2\), lactate and pyruvate, and the CSF lactate/pyruvate ratio. A small, but statistically insignificant increase in the average CSF lactate/pyruvate ratio was observed, which indicated that significant cerebral hypoxia\(^{8}\) was not present. Cardiac blood gases were generally within acceptable limits. All animals in this study were alert and moving about in a relatively normal fashion prior to each anesthesia suggesting that the arterial blood gases reflect acute changes related to anesthesia and not an equilibrium situation. Sugi, et al.\(^{22}\) found a small increase in L/P ratio in hemorrhagic dog CSF. Calculations using the data of Granholm\(^{4}\) give a value of approximately 2 mEq/L lactate produced in four hours in the case of subarachnoid hemorrhage, based upon 1 Gm% recovered total rbc counts produced over the first four hours for the rabbit is comparable to these results, since our recovered total rbc counts averaged 467,000 rbc/mm\(^3\) to 1 Gm% hemoglobin in the rabbit which would represent approximately 477,000 rbc/mm\(^3\).

Our results suggest that the finding of a significant elevation of CSF lactate in hemorrhagic fluid with a normal or only slightly elevated CSF \(1/_{P}\) ratio may be taken as presumptive evidence of early subarachnoid hemorrhage. These findings should be most applicable in the range of four–six hours after onset; i.e. well before the expected predominance of bilirubin at approximately one–three days after subarachnoid hemorrhage.\(^{1, 9, 20}\) Note that a significant concentration of bilirubin did not occur in any of our samples. The applicability of these results to the clinical setting is being investigated in our laboratory. In the meantime, we share the opinion of others that within the period of time prior to the appearance of a significant concentration of bilirubin in the CSF, the most reliable and clinically feasible method of distinguishing traumatic lumbar puncture from subarachnoid hemorrhage is the determination of rbc counts or total hemoglobin concentrations\(^{20, 24}\) in serially collected CSF samples.

### References

13. Sigma Tech Bulletin 726 — U V

### Table 4

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<tr>
<th>Rabbit number</th>
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<td>Mean (7 Animals)</td>
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Angiographic Spectrum of Cervical and Intracranial Fibromuscular Dysplasia

Anne G. Osborn, M.D. and Robert E. Anderson, M.D.

SUMMARY Cephalocervical or intracranial fibromuscular dysplasia (FMD) can be identified by its characteristic angiographic appearance. Most of these lesions occur adjacent to the Cl-2 interspace, characteristically sparing the origins and proximal segments of the major extracranial vessels. Approximately 65% of our patients had bilateral involvement of the cervical internal carotid arteries. Thirty percent were associated with one or more intracranial aneurysms. The vertebral arteries were involved in 10% of the cases. Twenty-four of 25 cases were associated with symptoms of either subarachnoid hemorrhage or focal cerebral ischemia.

FIBROMUSCULAR DYSPLASIA (FMD) is a nonatheromatous angiopathy of unknown etiology. Originally thought to involve only the renal arteries, FMD has been identified in a large number of small and medium-sized arteries and — most recently — in the renal and mesenteric veins.

Cephalocervical arterial fibromuscular dysplasia is relatively uncommon, usually affecting only the extracranial internal carotid and vertebral arteries. Intracranial FMD is rare, with only sporadic cases reported in the literature. In many of these reported cases FMD was limited to the intrapetrosal internal carotid artery or involved only the carotid siphon. Hence, true intradural intracranial FMD is distinctly uncommon.

We present a series of 25 patients illustrating the broad spectrum of angiographic findings in cephalocervical fibromuscular dysplasia. Included in the series are four cases of true intracranial FMD. Serious clinical sequelae are often associated with this angiopathy.

Patients

Twenty of the 25 patients were females. Their ages ranged from four to 71 years, with an average age of 45.6 years. All but one patient presented with cerebrovascular symptoms indicating significant clinical disease (table 1). Ten of the 25 patients had transient ischemic attacks and six had definite cerebral infarctions. Nine patients had had documented episodes of subarachnoid hemorrhage. All angiograms were selective studies and were performed via the transfemoral approach.

Angiographic Findings

The angiographic findings are summarized in table 1. Seventeen of the 25 patients had isolated cervical FMD affecting one or both internal carotid arteries, while an additional patient had both cervical and intracranial disease. Two patients had diffuse FMD affecting both vertebral arteries and the left internal carotid artery, while four additional patients had FMD affecting all four vessels. The involvement was bilateral in approximately 65% of all cases where both internal carotid arteries were examined. Thirty percent were associated with intracranial aneurysm.

Cervical fibromuscular dysplasia

With one exception all cervical lesions were in the midportion of the internal carotid or vertebral artery adjacent to the first and second cervical vertebrae. The common carotid bifurcation and proximal internal carotid artery were spared in all these patients.

An angiographic pattern of multiple arterial dilatations separated by irregularly spaced concentric stenoses was found in eighteen (fig. 1A, B). An additional patient demonstrated this characteristic pattern in one internal carotid artery while smooth tubular stenosis, involving a long segment, was found in the contralateral internal carotid artery (fig. 2). The angiographic diagnosis of tubular FMD was documented at surgery in this artery.

One patient had typical FMD in one cervical internal

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