Multivariate Analysis of Plasma Enzyme Profiles in Severe Head Injury

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We followed the changes in the activities of four enzymes (aldolase, aspartate aminotransferase, creatine kinase, and lactate dehydrogenase) in plasma for four days after head injury. The progression of the changes differs significantly between survivors and nonsurvivors. Stepwise discriminant analysis, involving the four enzymes, allowed us to divide 81 to 92% of head-injured patients (n = 280 selected without conscious bias) correctly into the two groups as early as 72 hours after trauma. Most of the patients who were misclassified according to our biochemical criteria had received phenobarbital for sedation. Valuable prognostic information in head injury evaluation may thus be obtained by daily determination of enzymatic activities of these four enzymes.

Additional Keyphrases: aldolase · aspartate aminotransferase · creatine kinase · lactate dehydrogenase· discriminant analysis

The several clinical signs now used in the prognosis of head injury are characterized by their limited value (1). Thus we have looked for biochemical criteria that can be related to the clinical course after head injury.

Several studies (2–4) have involved multivariate analysis to see if the prognostic value could be improved. We also used this approach to try to discriminate head-injured patients into survivor and nonsurvivor groups. Having done so on a learning sample, we next verified this test on another head-injured population, the "testing sample."

Materials and Methods

Study population: The total number of cases was 346. The learning sample (L.S.),2 66 patients with clinical complications (see below, "Clinical signs"), was studied so that we could establish discriminant functions between survivors (n = 55) and nonsurvivors (n = 11). The testing sample (T.S.) consisted of 280 patients, selected without conscious bias, who were admitted for head injury with loss of consciousness.

Biochemical determinations: From all the patients, blood samples were collected with lithium heparinate anticoagulant at the time of admission and then daily during the first four days of hospitalization. However, we could not always maintain a rigid time schedule and some blood samples were missed. Enzyme activities were measured daily. If a delay in measurement was necessary, samples were centrifuged, the pellet was discarded, and the plasma was stored at 4°C.

Plasma ALD, ASAT, CK, and LD were determined at 30°C with an ENI-GEMSAEC centrifugal analyzer (Electro-Nucleonics, Inc., Fairfield, NJ 07006). "Optimized" kits were purchased from Boehringer Mannheim Corp. for ASAT (cat. no. 124362) (5), CK (cat. no. 181188) (6), and LD (cat. no. 124885) (5). For ALD, we used an adapted reagent (cat. no. 123838) (7). Results are given in IUB units (U) per liter.

Clinical signs: All the L.S. patients had severe head injury with loss of consciousness and clinical complications (coma, skull fracture, intracranial edema, and/or hematoma). The clinical signs for the T.S. patients (Table 1) were more intensively studied and were used to try to explain possible misclassification.

Statistical and prognostic analysis: For the L.S. we first used Student's t-test (8). The differences between the two groups were significant when p < 0.05. We then used the

### Table 1. Clinical Data Collected for Test Sample

<table>
<thead>
<tr>
<th>head injury date</th>
<th>Past history</th>
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<tbody>
<tr>
<td></td>
<td>• Alcoholism</td>
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<td></td>
<td>• Heart disease</td>
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<td></td>
<td>• Other diseases</td>
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**Diagnosis**

- Skull fracture
- Polytraumatism
- Intracranial edema
- Intracranial hematoma

- State of coma: I, II, III, IV

**Development**

- Phenobarbital sedation
- Death within five days because of head injury
- Death within five days for other reason
- Later death (more than five days after trauma), because of head injury, or because of other reasons

1. Nearly unconscious; II, unconscious with appropriate movements but only upon painful stimuli; III, unconscious with inappropriate movements; IV, no reaction.

2. Phenobarbital sedation according to Gobiet (16).

Fig. 1. Progression of the four enzymatic activities for survivors (left, n = 55) and nonsurvivors (right, n = 11) in the learning sample group.

Difference between survivor and nonsurvivor groups significant (+) (p < 0.05); ordinate: multiples of the median value (M). Δ. ALD; δ, ASAT; φ, CK; Ο, LD. Adm., time of admission; Laboratory median values (U/L) for healthy subjects:

ALD 4, ASAT 20, CK 70, LD 274.
program BMDP 7M (9) for stepwise discriminant analysis.

Results

For the L.S. patients, the enzymatic variations during the four days differed according to their clinical progression (Figure 1). When the outcome was favorable, the plasma enzyme activities were stable or decreased with time. In fatal cases, the enzyme activities continued to increase until death. We observed significant differences as early as 48 h for ALD and LD, but only 96 h after the trauma for all of the four enzymes. To test the prognostic value as to survival or death, we used a stepwise discriminant analysis (BMDP 7M program) with the following four parameters. First, we calculated the cumulative probabilities for discriminating the two groups, taking into account ASAT, ASAT + CK, ASAT + CK + ALD, and ASAT + CK + ALD + LD (Table 2). Next, we calculated discriminant functions from the activities of these four sets of enzymes at 72 h and 96 h after injury. The validity of the functions based on the last set was blindly tested with the T.S. population (Table 3) and evaluated in terms of sensitivity (well-predicted survivors), specificity (well-predicted nonsurvivors), and efficiency (total of well-classified patients) according to Galen and Gambino (10) and Henderson and Nealon (11).

Discussion

We chose to study enzymatic activities because of their rapid variations in plasma. We preferred to use plasma rather than cerebrospinal fluid because successive samplings would be without risk to the patient. Cerebrospinal fluid sampling may be dangerous in intracranial edema.

Activities of these enzymes considered separately are not specific for head injury. In fact they also are used to detect cardiac diseases (12, 13, 14) or muscular diseases (15). However our results demonstrated that, combined, they may increase the predictive value of the clinical progression or outcome of a head injury (Table 2). Activities of these four enzymes—but not of alkaline phosphatase, a-amylase, γ-glutamyltransferase, glutamate dehydrogenase, or alanine aminotransferase—significantly differed between survivor and nonsurvivor groups some time after the trauma. However, changes in ALD and LD seem to appear earlier than those in ASAT or CK. Thus, the prognostic value of each enzyme may differ but combining such information may increase its prognostic value. By trying discriminant analysis, so as to have the most efficient information with the minimum of biochemical determinations, the efficiency of our approach was fairly good. In fact, we found that discrimination was better at 72 h than at 96 h for the four variables combined.

We also observed that head injury without clinical complication does not increase the activities of these enzymes in plasma (unpublished data).

Inclusion of ALD activity significantly increases the efficiency of discrimination. Further studies are now under way to examine whether it is a more specific enzyme for head injury.

Table 4 lists the main clinical characteristics of misclassified patients. We observed that phenobarbital sedation according to Gobiet (16) was a common feature in survivors whose enzyme data indicated an unfavorable prognosis, although we cannot affirm that this therapy changed their clinical course. Those patients who died despite a favorable enzymatic prognosis often had intracranial disorders (edema or hematoma). More cases must be studied to elucidate the origins of misclassification.

Concentrations of acute-phase proteins (especially C-reactive protein) in serum increase quickly and significantly in head injury complicated with edema or hematoma (17, 18). Although this protein is not specific for cerebral edema or hematoma, such data added to the data on enzymatic activities might increase the efficiency of prognosis. We also have further studies under way to examine this possibility.

Do these enzymatic activities come from cerebral or peripheral cells? For CK, Jung and Ferard (19) showed that, in trauma cases, the BB isoenzyme represented only 2% of the total CK activity in plasma; most was the MM isoenzyme, which is mainly concentrated in muscle cells. The mechanism of this enzyme release is still to be explained.

We conclude that, when these four enzymes’ activities are measured, the prognosis for head-injured patients can be made more reliably. We could distinguish survivors from nonsurvivors with an efficiency ranging between 81 and 92%. We believe that these activities should be measured daily and compared with other biochemical or clinical data for the greatest efficiency.

References


