Clinical and biochemical predictors of late-outcome in patients after ischemic stroke

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INTRODUCTION

The possibility of prognosis of long-term outcome in people affected by ischemic stroke (IS) is a matter of great importance for patients, their caregivers and doctors working in stroke units (SU). SU patients are hospitalized for a defined, rather short period of time. Accurate prognosis concerning the outcome can help to plan the rehabilitation process and determine the need for care of caregivers. Many factors including demographical features, clinical scales and biochemical parameters have been assessed until the present according to their usefulness as predictors [1, 2, 3].

The National Institute of Health Stroke Scale (NIHSS) is a very popular scale for scoring neurological deficits by assessing 11 neurological deficits and their degree of intensity. The final score can vary from 0–42 points. It is also considered a valuable tool for measuring long-term outcome after stroke IS [4, 5, 6]. Additionally, analysis of the NIHSS score is used as a method for assessing the efficacy of the services for IS patients [7] and has been widely used in clinical trials [8].

There are also other widely used scales for functional assessment, such as the Barthel Index (BI) and the modified Rankin Scale (mRS). In 1965, BI was offered as modification of the ‘Maryland disability index’ and began to be used as a valuable tool for assessing improvement in rehabilitation [9]. The scale describes 10 tasks. Each task is scored according to the age of patients [13, 2]. The measure of initial infarct size is a strong and independent predictor of IS outcome in long-term observations [14, 15].

Conclusions. BI assessed on day 10 has a predictive value for the outcome evaluated by mRS 3 months after the onset of IS.

Key words

CRP, outcome, Ischemic stroke, MRS, Barthel index, NIHSS.
Numerous studies have been devoted to discover biochemical markers of the neurological diseases that could be useful as diagnostic or prognostic indicators. These substances are released into the cerebrospinal fluid (CSF) [24] and later into the bloodstream during different processes of ischemia [24, 25]. S100BB protein is a well-known glial cell marker and its increased level can predict unfavourable outcome after IS [1, 26, 27]. Tau protein, released from axons during ischemia, has also become an object of interest as a predictive factor [28, 29] and therapeutic target for IS [30].

**OBJECTIVE**

The aim of the prospective study is to evaluate different clinical scales, biochemical and radiological parameters measured on day 10 after the stroke onset, according to their value as predictors of long-term outcomes. The day of measurement was chosen as a mean stay at SU in 10 days. As the endpoint, the mRS score after 90 days since the onset of IS was chose. The mRS has some limitations but it is a globally used scale, clearly and directly indicating the possibility of independent functioning of patients after IS in their daily environment. Global disability on days 7–10 after suffering IS strongly predicts the final 3-month disability outcome [31]. Additionally, previous studies have proved its high sensitivity as the end point for detecting treatment effect [32].

**MATERIALS AND METHOD**

Sixty patients admitted to the Stroke Unit in the Department of Neurology of the Medical University in Lublin, Eastern Poland, were prospectively enrolled into the study. All patients (or their family members) received full written and oral information regarding all study procedures, and signed an informed consent. The Ethics Committee of the Medical University in Lublin approved the protocol and the informed consent. Inclusion criteria were: (a) diagnosis of IS based on history, physical examination and computed tomography (CT) performed upon admission to the hospital, (b) admission to the hospital within the first 24 hours of the onset of neurological focal symptoms. Exclusion criteria were: (a) regression of neurological symptoms within 24 hours of the onset (Transient Ischemic Attack, TIA), (b) previous history of central nervous system diseases, (c) time of hospitalization shorter than 10 days, (d) haemorrhagic transformation of ischemic focus, (e) recurrent stroke. All patients did not fulfill the criteria for r-tPA treatment. The final study group consisted of 45 patients. Rehabilitation was applied to all patients which consisted of passive or active exercises, according the patient's status, 30–40 minutes once a day.

**Neurological Assessment.** Neurological examination was performed on day 1 and day 10 after admission to hospital, based on the scales: NIHSS, BI and mRS. After 3 months, 38 patients were examined according to BI and mRS by phone. 7 patients died within 3 months of their discharge from hospital.

**Imaging studies.** A CT-scan was performed on day 10 day (additionally to that performed on admission) of hospitalization, without contrast, using a 64-row multidetector CT (LightSpeed VCT with workstation Advantage Window 4.3). The volume and mean density of ischemic foci were measured by means of the planimetric method, with the use of an additional workstation for measuring infarct volume in 3D.

**Biochemical procedures.** Venous blood samples were obtained during the first 24 hours after stroke and on day 10 day onset of symptoms (fasting specimens at the same time in the morning). After centrifugation, sera was stored at -60°C for a maximum period of 8 months. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to evaluate serum S100BB (CanAg Diagnostics AB, Gothenburg, Sweden), Tau protein (Innotest, Innogenetics NV, Gent, Belgium), CRP (R&D Systems Inc.Minneapolis, USA) and DD levels (Innovance, Siemens Health Diagnostics Products GmbH, Marburg, Germany), according to the manufacturer’s instructions. Detection limit of S100BB and Tau protein was 10 pg/mL and 60 pg/mL, respectively. All values below detection limit were rendered zero and not used in calculations. Optical density was determined by use of a microplate reader set at 450 nm. The cut-off value for DD was 500 μg/L. Measurement of albumin serum level was based on electrophoretic methods.

**Statistical analysis.** Statistical analysis was performed with the use of the SPSS (Statistical Package for Social Sciences) software for Windows (Version 20.0). The average standard deviation and percentiles were provided for descriptive analysis. Statistical differences between non-dependent groups were calculated using the Mann-Whitney U test and the χ² test. Wilcoxon signed ranks test was used to compare the two dependent groups. The Spearman rank correlation test (R) was employed to search for correlation between the functional status and levels of CRP, albumin, DD, S100BB and Tau proteins, and volume of ischemic focus. Logistic regression modeling was used to identify predictors of good and bad outcomes 3 months after stroke. p<0.05 was accepted as statistically significant. Multiple logistic regression using the backward stepwise method was applied, and for the criterion of removal variables, the test of likelihood ratio was used. The probability of removing variables was 0.06. All explanatory variables introduced into the model were quantitative.

**RESULTS**

Ultimately, 45 patients with IS were evaluated: 23 women and 22 men, of whom 27 (60%) were atherosclerotic, 10 (23%) cardioembolic and 7 (16%) were of undefined etiology. Patients were between 50–93-years-old. The average age was 72.42 (SD=11.18).

Significant correlations were observed between NIHSS and BI evaluated on day 10 of IS, and mRS 3 months after IS onset (R= 0.533; p< 0.001, R= -0.525; p<0.001, respectively). Positive correlation between volume of ischemic focus measured on day 10 of IS and the late outcome was also noted (R=0.45; p<0.01). Among the biochemical factors which can influence IS outcome and were evaluated on day 10 of IS, CRP and albumin levels, but not DD level, correlated with results of mRS after 3 months (R= 0.42; p<0.01 and R= 0.41; p<0.05 for
CRP and albumin, respectively). There were no significant correlations between S100BB and levels of Tau proteins on day 10 and mRS 3 months after onset of IS.

Patients were divided into two groups in terms of results of their late outcome (90 days after stroke onset). The first group was with good outcome (GO) and consisted of patients without neurological deficits or with disability that did not affect their independence (mRS 0–2). The second group was with bad outcome (BO), and consisted of patients dependent on others or of those who died (mRS 3–6).

13 people (28.88%) were in the GO and 32 (71.22%) in the BO group (including 7 who died).

There were no statistical differences between the two groups in terms of age and gender. There were more patients with atherosclerotic etiology in GO than in BO (p = 0.02).

**Table 2.** Comparison of demographic, clinical and biochemical characteristics in patients with good (mRS 0–2) and bad (mRS 3–6) outcome 3 months after ischemic stroke (Mann-Whitney U test or χ² test).

<table>
<thead>
<tr>
<th>Variable</th>
<th>GO (mRS 0–2)</th>
<th>BO (mRS 3–6)</th>
<th>U/χ² test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>68.69 (9.19)</td>
<td>73.94 (11.7)</td>
<td>1.443</td>
<td>0.156</td>
</tr>
<tr>
<td>Male/female</td>
<td>9/4</td>
<td>13/19</td>
<td>3.027</td>
<td>0.082</td>
</tr>
<tr>
<td>Etiology of IS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– atherosclerosis</td>
<td>0</td>
<td>10</td>
<td>7.856*</td>
<td>0.02</td>
</tr>
<tr>
<td>– cardioembolism</td>
<td>12</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– undefined</td>
<td>1</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of ischemic focus (mL)</td>
<td>6.23 (8.11)</td>
<td>54.38 (80.28)</td>
<td>-2.542*</td>
<td>0.011</td>
</tr>
<tr>
<td>NIHSS on day 10</td>
<td>4.54 (6.06)</td>
<td>9.59 (5.47)</td>
<td>-2.875**</td>
<td>0.004</td>
</tr>
<tr>
<td>Barthel Index on day 10</td>
<td>76.54 (32.81)</td>
<td>29.84 (34.56)</td>
<td>-3.316***</td>
<td>0.001</td>
</tr>
<tr>
<td>S100BB (pg/mL) on day 10</td>
<td>43.40 (20.73)</td>
<td>45.16 (29.58)</td>
<td>-0.068</td>
<td>0.946</td>
</tr>
<tr>
<td>Tau (pg/mL) on day 10</td>
<td>20.62 (40.48)</td>
<td>57.67 (100.39)</td>
<td>-1.075</td>
<td>0.283</td>
</tr>
<tr>
<td>CRP (μg/mL) on day 10</td>
<td>12.80 (14.21)</td>
<td>31.39 (29.23)</td>
<td>-2.378*</td>
<td>0.017</td>
</tr>
<tr>
<td>D-dimer (μg/L) on day 10</td>
<td>1550.54 (1902.97)</td>
<td>1328.27 (1252.26)</td>
<td>-0.244</td>
<td>0.088</td>
</tr>
<tr>
<td>Albumin (g/dL) on day 10</td>
<td>3.80 (0.97)</td>
<td>3.69 (0.43)</td>
<td>-1.640</td>
<td>0.101</td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.01; *** p<0.001
with ischemic stroke (IS) [21, 22]. However, earlier studies took into account the albumin level measured on the first day, and not on day 10 of IS [21].

The value of the DD level as a prognostic factor for long-term outcome after IS is controversial [23, 38]. The results of the current study support the opinion of those authors who failed to consider DD level as an indicator of late outcome after IS [38]. Specific substances of the nervous tissue which are released into the blood during IS are of great interest as potential predictors. The authors of this study found no correlations between S100BB and Tau proteins, and late outcome. S100BB protein reached a peak level on day 3 of IS; therefore, its evaluation on day 10 showed the limitation of its usefulness as a predictor for late outcome of IS. Tau protein was found in only 43.9% of patients in the current study, which makes that protein less valuable as a predictor. These results confirm those of a previous study [29] which indicated that detection of Tau protein in the serum of patients with IS, but not its concentration, can be considered as a bad prognostic factor for the clinical outcome in the early and late phases of IS.

The functional status of a patient after stroke is also influenced by factors such as mental status and comorbidities or nutritional status. The neurological deficit caused by IS overlaps the pre-morbid functional status. For this reason, functional (BI), but not biochemical assessment measured on the day of discharge from hospital, is a better predictor of the long-term outcome.

The limitation of this study was the small sample of enrolled patients. This can account for the fact that the results obtained did not consider demographic factors, such as age and gender as important for post-IS prognosis, which is contrary to the results of other authors [39, 40]. Evaluation of clinical scales was performed at a hospital, which may influence the results due to the possible limitation of a patient’s activity by members of staff. However, the presented results are statistically significant which is an important and valuable observation which could direct and inspire further clinical researches.

CONCLUSION

Among different clinical scales, biochemical parameters and radiological findings, the most useful in long-term prognosis is clinical assessment by BI. BI assessed on day 10 has a predictive value for the outcome evaluated by means of mRS 3 months after the onset of IS.

REFERENCES