SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

PROSPECTIVE EXAMINATION OF FACTORS INFLUENCING LONG-TERM STROKE OUTCOME WITH STATISTICAL METHODS IN THREE STROKE CENTERS

Klára Edit Fekete MD

Supervisor: Dániel Bereczki MD, DSc

UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2014.

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By: Klára Edit Fekete MD

Doctoral School of Clinical Medicine, University of Debrecen

Supervisor: Dániel Bereczki, MD, DSc

Head of the Examination Committee: András Berta, MD, DSc

Members of the Examination Committee:
Péter Diószeghy, MD, PhD
Norbert Németh, MD, PhD

The Examination takes place at the Library of the Department of Ophthalmology, Faculty of Medicine, University of Debrecen, 23 rd of April 2014, 11:00 AM.

Head of the Defense Committee: András Berta, MD, DSc

Reviewers: Sándor Horváth, MD, PhD
Sándor Szabó, MD, PhD

Members of the Defense Committee:
Sándor Horváth, MD, PhD
Sándor Szabó, MD, PhD
Péter Diószeghy, MD, PhD
Norbert Németh, MD, PhD

The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 23rd of April 2014, 13:00.

1. INTRODUCTION AND BACKGROUND

Cerebrovascular diseases are the most common neurological disorders. According to the assumption of the World Health Organisation in 2002 there were 15.3 million strokes, and one third of them (5.5 million) had fatal outcome. According to the international data in 2010 the incidence of stroke was 16.9 million, due to stroke 5.9 million deaths were reported and the lost-DALY (Disability Adjusted Life Year) was 102 million. In Hungary the incidence of stroke is higher than in Western-European countries.

The rate of severe disability is high among the survivors of stroke (ischaemic stroke 62%, haemorrhagic stroke 78%), which emphasizes the economic and social importance of the disease. The burden of stroke is expected to become more pronounced with the aging of the population, and with a higher ratio of severely disabled patients.

Nowadays there are safe and effective treatments (recombinant tissue plasminogen activator /rtPA/) for patients with acute stroke transferred in time to stroke centers. Although the mortality of haemorrhagic stroke is still high, according to guidelines treatment in stroke centers, neurosurgical intervention in case of specific conditions may decrease the rate of fatal outcome.

Several studies examined factors that may be responsible for stroke, giving a tool for primary prevention. It is also important to find the predictors influencing outcome after stroke, however, studies on long-term outcome are rare.

2. AIM OF THE STUDY

The incidence of stroke is higher, the short and long-term outcome is worse in the Central-Eastern-European region than in Western-European countries. Therefore we set forth to examine the association of some known stroke risk factors (smoking, alcohol-consumption) as well as mannitol and rtPA as acute stroke therapies with outcome.

I. We assumed, that the higher rate of smoking and chronic alcohol consumption might have an impact on stroke mortality not only by being risk factors for stroke, but also by increasing stroke severity. We aimed to test whether prestroke chronic alcohol-consumption and smoking are associated with more severe stroke signs and worse outcome by testing this hypothesis in 3 Central-Eastern European countries (Hungary, Romania, Ukraine), in a database containing more than 1000 patients' data.

II. The clinical efficacy of mannitol in acute stroke is unclear. In Ukraine mannitol is rarely used in acute stroke management, while in Romania and Hungary mannitol is more or less frequently applied. Therefore we aimed to test if there is any association of outcome with mannitol treatment status in patients with strokes of similar severity.

III. We prospectively registered patients treated with rtPA in the Debrecen thrombolyis database. Approximately 600-700 acute stroke patients were treated annually from the catchment area of 600 000-700 000 inhabitants. Although the ratio of thrombolysed patients is similar in the last few years to that in Western-European countries'(16-18%), long-term outcome seemed worse in Hungary. Therefore we set forth to find factors that are independent predictors of long-term outcome after thrombolysis.

3. METHODS AND PATIENTS

We established and used two databases in our studies. The *Mures-Uzhgorod-Debrecen (MUD) database*, a prospective follow-up study was used to evaluate the association of alcohol-consumption, smoking and mannitol treatment with stroke outcome.

The MUD database contains altogether 1049 patients' data (603 male, 446 female). All participating centers are tertiary stroke centers at the Departments of Neurology, University of Debrecen, Hungary (210 000 inhabitant, 554 patients), University of Targu Mures, Romania (165 000 inhabitant, 261 patients) and University of Uzhgorod, Ukraine (126 000 inhabitant, 234 patients). We developed a standard case report form in the languages of the participating countries included age, gender, risk factors, neurologic status at admission by stroke scales, and treatment on ward, functional performance at discharge, and survival at 30 days and 1 year.

We established the prospective *Debrecen Thrombolysis Database*, and used this database to examine factors that might influence short- and long-term outcome in patients treated with rtPA. The database consists all 415 patients' data treated with rtPA at the Department of Neurology, University of Debrecen between the 1st of January 2004 and 31st of December 2010. The catchment area of the center is 90 km with approximately 600 000 inhabitants, and 600-700 acute stroke admissions per year.

3.1. The association between alcohol consumption, smoking and stroke outcome

Patients

We analyzed the Mures-Uzhgorod-Debrecen database. Data of all patients consecutively admitted with acute cerebrovascular disease between October 1,

1999 and September 30, 2000 to the 3 centers were prospectively entered into the database. The admission National Institutes of Health Stroke Scale (NIHSS) score was used. Based on computed tomographic (CT) examination (88.5% of cases) and the clinical characteristics, the current acute cerebrovascular disease was categorized as ischaemic stroke, transient ischaemic attack, intracerebral haemorrhage (ICH), and subarachnoid hemorrhage. For discharge condition, outcomes of the International Stroke Trial were used with a minor addition, that is, died or survived; if survived, completely recovered or had residual signs; in the presence of residual signs, independent or dependent; and if dependent, does the patient need continuous care or needs help only in some activities. There were follow-ups at 30 days and at 1 year after stroke to identify survival status by personal visits, questionnaires sent by mail or telephone calls of the patients, their relatives, or their family practitioners.

Alcohol consumption and smoking were self-reported at admission. Obtaining reliable answers regarding alcohol consumption and smoking habits is difficult; therefore, we categorized patients only as nonregular drinkers (included nondrinkers and occasional light drinkers: 25 g/day) and regular drinkers (more than 25 g/day). As only a few patients were alcoholics according to the World Health Organization definitions, these patients were merged into the regular drinker group. Because the results during the exploratory analyses were the same whether the patients smoked 1-10, 11-20, or more than 20 cigarettes a day, in the final analyses, patients were divided only into smoker and nonsmoker groups. We excluded from the main analysis those who had missing data about their drinking or smoking habits, but included them in worst case scenario sensitivity analyses.

Statistical Analysis

Variables were described using standard statistics. Associations between categorical variables were evaluated by chi-square tests. Effects of factors on the

odds of death at 30 days and 1 year were estimated using multiple logistic regression and were expressed as odds ratios with 95% confidence intervals (CIs). Age and gender were used in interaction after exhausting other interaction possibilities. Explanatory variables used included the a priori factors of alcohol use and smoking status and those found to possess significant effects and/or importance in elimination of confounding. Model fit was checked using the Hosmer–Lemeshow test. The statistical package Stata (version 10) was used for the analysis.

3.2. The association between mannitol therapy and stroke outcome

Patients

The database of the Mures-Uzhgorod-Debrecen study was analyzed. The database includes information on risk factors; patient condition on admission, including prognostic and long-term items of the Scandinavian Neurological Stroke Scale (SNSS); treatment on the ward; and condition at discharge. For discharge condition, outcome according to the International Stroke Trial was used, with a minor addition. Follow-up was performed at 30 days and 1 year after stroke by personal visits, postal questionnaires, or telephone calls to the patients, their relatives, or their family practitioners. From the current analysis, we excluded those with transient ischaemic attack, cases of subarachnoid hemorrhage, and those who were admitted after 72 hours of stroke onset.

The database was originally designed for epidemiological and audit purposes and not to test treatment effects in a controlled fashion; therefore, some information important for the present analysis had to be additionally obtained. Such information was extracted from the patients' documents retrospectively for this analysis. The following factors were considered in the analysis: age; prestroke dependency; time to admission from stroke onset; diabetes; previous stroke, malignancy, and peripheral arterial disease in the history; smoking status;

serum glucose level on admission; disturbance of the level of consciousness (LOC) on admission; the prognostic and long-term scores according to the SNSS (smaller SNSS scores indicate more severe strokes); white cell count in the first 3 days after admission; fever in the first 3 days after admission, defined as axillary temperature >37°C; fever at any time during the hospital stay; chronic obstructive pulmonary disease; atrial fibrillation during the hospital stay; antibiotic use, aspirin treatment, and heparin treatment during hospitalization; respirator use other than during attempts of resuscitation; and nasogastric tube feeding.

Statistical analysis

Continuous variables were compared by ANOVA. Stroke scale scores were compared by the Mann-Whitney test. The Pearson chi^2 test was used to compare frequencies. Logistic regression models were used to evaluate whether 30-day and 1-year case fatalities depended on mannitol treatment status. In the models, survival was the dependent variable, and those factors that were found to be different by univariate analyses between the treated and nontreated groups were entered as continuous predictors or categorical factors. We used Statistica for Windows, version 6.1 (StatSoft) and the Proc Logistic procedure of SAS, version 8.02 (SAS Institute).

3.3. The predictors of outcome after intravenous or intraarterial thrombolysis

Patients

Between the 1st of January 2004 and the 31st of December 2010, 415 patients were treated with rtPA at the Department of Neurology, University of Debrecen. According to actual guideline recommendations, for IV treatment the time window from symptom onset was 3 hours until October 2008, and 4.5 hours afterwards. Cases where treatment indication did not follow the guidelines

were excluded and 369 patients' data were analyzed. Four patients were lost to long-term follow up.

Database

A database was created recording age, gender, time of stroke onset, time of arrival to the hospital, time of the CT scan result, time of administration of rtPA, previous medical history (hypertension, diabetes mellitus, atrial fibrillation, heart failure, self reported smoking and alcohol consumption habits), on-admission stroke severity (including the National Institute of Health Stroke Scale Score - NIHSSS), prestroke modified Rankin Scale score (mRS), systolic and diastolic blood pressure before the initiation of rtPA administration, serum glucose, INR, APTT, cholesterol, triglyceride, on-admission CT/CTA scan, if performed digital subtraction angiography and a follow-up (24 hours ± 2 hours) CT scan. We registered the NIHSS Score at 24 hours, medications for secondary prevention, mRS at 3 months, and survival status at one-year.

Imaging

A non-contrast computed tomography (CT) was performed on admission. Arterial occlusion (trunk or at least 1 branch of any large artery) was identified by CTA. CT was repeated one day after treatment and in case of clinical deterioration. The Alberta Stroke Programme Early CT Score (ASPECTS) was determined unblinded to patient characteristics and was stratified to ≤7 (group I-severe) and above 7 (group II-mild). Haemorrhagic infarction or parenchymal haematoma were defined according to the European Cooperative Acute Stroke Study. We used three definitions for symptomatic intracerebral hemorrhage (SICH): the SITS, the ECASS and the NINDS criteria.

Treatment

IV rtPA treatment was the standard practice, and administered according to guidelines. Of these 202 cases with CTA-proven vessel occlusion IA thrombolysis was performed in 46 patients, and in 12 cases treatment started IV

and was followed by IA administration ("bridging" therapy). For IA intervention repeated doses of 5 mg rtPA was given, until opening of the artery or the maximum IV dose was reached. The decisions were individually brought by the treating physician who consulted with the radiologist.

Outcomes

The NIHSSS and case fatality were evaluated the day after admission. The mRS was used to assess outcome at 3 months and dichotomized to favorable (mRS 0-2) and unfavorable (mRS>2 points). Survival status was evaluated at one year.

Statistical analysis

Statistical analysis was carried out using the SPSS for Windows 19.0 (SPSS Inc. Chicago, USA). Categorical variables were assessed with Pearson χ^2 test. Binary logistic regression analysis was used to assess outcome at 3 months and at one year.

Logistic regression models were used to identify the independent predictors of 3-month disability and 1-year case fatality. The analysis was preformed with the multivariate general linear model (GLM). In the models, disability at 3 months (mRS >2), and case fatality at one year were the dependent variables, and those factors that were found to be associated with outcome by univariate analyses were entered as confounding variables. In the multivariate analysis we used the following NIHSSS categories: 0-7, 8-14, 15-22, >22. The variables were excluded from the analysis one by one, and the variable with $p > 0.05$ and closest to 1.0 dropped out, until all features left in the model had $p < 0.05$.

4. RESULTS

4.2. The association between smoking and alcohol consumption and stroke outcome

4.2.1. Distribution of patients by stroke type, smoking, and alcohol consumption

Twenty patients (1.9%) had other confounding diagnoses. Those with subarachnoid hemorrhage (n= 546, 4.4%) were excluded, whereas those with transient ischaemic attack were included in the ischaemic stroke group in data analyses. Alcohol consumption was admitted by 24.7%, whereas 54.7% claimed themselves to be nonconsumer or light drinker, and there was no clear answer from 20.5%. The rate of alcohol consumers was significantly higher among men ($p<0.001$). Of the patients, 24.5% smoked. Smoking was 3 times more frequent among men than women (chi-square test, $P <0.001$). Clear information on smoking could not be gained in 10.4%.

4.2.2. The effect of smoking and alcohol consumption on stroke severity

In the total group, median stroke severity by the NIHSS score was 7 (minimum: 1, maximum: 33). Stroke was most severe, and case fatality was highest in ICH. The median NIHSS score was similar in the total group and in those with ischaemic stroke, in smokers and nonsmokers, and in alcohol consumers and nonconsumers/light consumers. We categorized strokes by the NIHSS score as mild (0-7), moderate (8-14), severe (15-22), and very severe (>23). There was no difference in the distribution of patients among these severity categories between smokers and nonsmokers ($p=0.71$) and alcohol consumers and nonconsumers ($p=0.65$). When the analysis was restricted to ischaemic strokes, stroke severity did not differ significantly between smokers

and nonsmokers. The proportion of cardioembolic strokes was also similar (21.5% among smokers and 18.4% among nonsmokers, p=0.35).

4.2.3. Disability at discharge

Hospital case fatality was 12.2%, at discharge 15.2% needed permanent care, 17.6% needed help with everyday life activities, 30.8% could live a self-sufficient life with residual signs, and 20.6% had no neurologic signs. In 35 (3.6%) survivors, accurate data were missing on the severity of disability. There was no significant association between prestroke smoking status and functional outcome in all cases, in ischaemic stroke patients, and in ICH (p> 0.13, p> 0.5, and p> 0.08). The number of smokers among ICH patients was small (n= 519); therefore, no firm conclusion can be made for the association of smoking and functional outcome in ICH. Regarding the association of alcohol consumption and functional activity at discharge, there was an unfavorable trend in haemorrhagic stroke in alcohol consumers (haemorrhagic stroke: p=0.1235; ischaemic stroke: p=0.6747).

4.2.4. Effects of gender, age, stroke type, and stroke severity on outcome

Of the cases, 42.5% were women with no significant difference among centers. At 30 days, case fatality patients at 30 days (OR 0.95, 95% CI: 0.53-1.73) and at 1 year (OR 1.34, 95% CI: 0.83-2.16) men and women were 16% and 17%, respectively (p =0.62). At 1 year, case fatality was 26% among men and 32% among women (p=0 .039). Below 51 years of age, 30-day case fatality was 7.7% in men and 13.5% in women (OR= 0 .11, 95% CI:0 .01-0.77, p=0.026), and 1-year case fatality in this age group was also significantly lower (men 10,2%, women 18,9%) in men (OR=0.18, 95% CI: 0.04-0.83, p=0.0275).

In the young age group (<51 years), we did not find any major differences between men and women in the examined risk factors and stroke severity on

admission. In the other age subgroups, there was no significant difference in the 30-day and 1-year case fatality between men and women.

When comparing case fatality by age groups separately in men and women, using cases below 51 years as the reference group, the odds of death at 30 days and at 1 year increases with age in men, whereas in women the odds of death at 30 days is similar across all age groups. The odds of 1-year case fatality increase with age in women as well, reaching statistical significance only in those over 80 years of age. ICH is associated with significantly higher death rates than ischaemic stroke (OR=4.85, 95% CI: 3.06-7.67, p=0 .0001 at 30 days; OR=3.47, 95% CI: 2.27-5.28, p=0.0001 at 1 year). The NIHSS score at admission is a strong predictor of both 30-day and 1-year outcome.

4.2.5. The effect of smoking and alcohol consumption on case fatality at 30 days and 1 year after stroke

Smoking did not result in significant increase in case fatality at 30 days (OR=1.41, 95% CI: 0.82-2.43) and at 1 year (OR=1.09, 95% CI: 0.69-1.71). When ischaemic strokes and cerebral hemorrhages were analyzed separately, there was no difference in 30-day case fatality between smokers and nonsmokers in ischaemic and haemorrhagic strokes. At 1 year, case fatality was similar in smoker and nonsmoker ICH cases, whereas in ischaemic strokes 1-year case fatality was 19.5% in smokers and 26.9% in nonsmokers (p=0.037). There was no significant difference among alcohol consumers and nonconsumers in case fatality in all stroke patients at 30 days (OR=0.95, 95% CI: 0.53-1.73) and at 1 year (OR=1.34, 95% CI: 0.83-2.16). Comparing alcohol consumers to nonconsumers, no difference in 30-day and 1-year case fatality was observed in ischaemic and in haemorrhagic strokes (p >0.2 in all comparisons), although there was a trend of increase in case fatality in alcohol

consumers in haemorrhagic stroke (39.5% vs 26.4%, p>0.2, at 30 days and 48.8% vs 35.8%, p>0.2, at 1 year).

High case fatality was observed in those with unknown alcohol consumption (29.7% at 30 days and 46.5% at 1 year) and in those with unknown smoking status (41.2% at 30 days and 58.8% at 1 year). More severe strokes in these groups could have prevented to obtain reliable information regarding smoking and alcohol habits.

4.3. The association of mannitol treatment with stroke outcome

4.3.1. Characteristics of patients

Of the acute stroke patients registered in the database, 805 were admitted within 3 days of stroke onset. Of these patients, 665 had ischaemic stroke. The mean±SD age was 66±12.5 years; there were 471 men and 334 women. Time from stroke to admission was <24 hours in 568 patients; of these, 457 had ischaemic and 111 had haemorrhagic strokes. Thirty-day and 1-year survival data were available for 782 of 805 (97%) and for 768 of 805 (95.4%) patients, respectively. The overall case fatality was 22.1% at 30 days and 33.6% at 1 year. Case fatality in the mannitol-treated subgroup was significantly higher both at 30 days (25% vs 16%, p=0.006) and at 1 year (38% vs 25%, p<0.001).

4.3.2. Characteristics of mannitol treatment

The rate of mannitol treatment significantly differed among the 3 centers but did not differ between sexes. Mannitol was given according to the discretion of the treating physician, and when given, the mannitol solution was administered intravenously for 3 to 10 days. The mean dose of mannitol was 47±22 g/day, and the mean duration of mannitol treatment was 6±3 days. Mannitol treatment was initiated on the day of admission in 97%, and only 3% of the patients received mannitol for worsening of their condition. Except for

mannitol, no other osmotically active medications (glycerol, hypertonic saline, urea, etc) were used by the centers.

4.3.3. Comparison of mannitol-treated and nontreated patients

All Patients

Mannitol-treated patients were older, stayed longer in hospital, and had somewhat lower SNSS prognostic and long-term scores than did those who were not treated with mannitol. More of the mannitol-treated patients were dependent before their current stroke. Artificial ventilation and nasogastric tube feeding were more frequent in mannitol-treated patients. Of the 666 patients with ischaemic strokes, aspirin was given to 72% of the mannitol-treated and to 50% of the nontreated patients ($p<0.001$).

Patients admitted within 24 hours after stroke

Mannitol use was >70% in those admitted within 24 hours, 56% among those admitted between 24 and 48 hours, and 31% among those admitted between 48 and 72 hours; ie, with a longer delay to admission, the application rate of mannitol significantly decreased ($p<0.0001$). Therefore, we performed a separate analysis in the subgroup of those 568 patients who were admitted within the first 24 hours after stroke onset, and within this group, 2 further analyses were performed for those with ischaemic and haemorrhagic strokes. Mannitol use was the same in ischaemic and haemorrhagic strokes.

The proportion of prestroke dependency was 3.6% in nontreated and 13.5% in mannitol-treated patients ($p <0.001$). Fewer patients in the mannitol group were smokers. Disturbance of consciousness on admission was more frequent in the nontreated group, whereas fever within the first 72 hours, respirator use, and nasogastric feeding was more common in the mannitol treated group. Although SNSS prognostic scores were similar, there was a tendency in the mannitol-treated group for a higher 30-day case fatality (23% vs 17%, $p=0.13$). The

marginally lower long-term SNSS score was associated with higher 1-year case fatality in the mannitol group.

4.3.4. Outcome

Outcome in all patients

Without considering other factors, mannitol treatment was associated with significantly decreased odds of 30-day and 1-year survival (p=0.005 and p=0.0002, respectively). When mannitol treatment status was adjusted for age and the presence of disturbance of consciousness on admission, mannitol still seemed to have an adverse effect (p=0.0028 for 30-day and p=0.0017 for 1-year survival). When the prognostic score of the SNSS (ie, the sum score of the LOC, eye movements, and arm and leg strength items; score range, 0 to 22) was used in the model instead of the presence or absence of disturbance of consciousness, mannitol treatment did not have a significant effect on survival (p=0.1931 and p=0.1241 for 30-day and 1-year survival, respectively). Case fatality at 1 year was 38% in the mannitol group and 25% in the nontreated group (p=0.0002).

Age, presence of a disturbance of consciousness on admission, and mannitol treatment were significantly associated with 1-year case fatality (p=0.002 for all). In an extended model, age (p<0.001), SNSS long-term score (p<0.001), and fever in the first 72 hours (p=0.011) were significantly associated with case fatality, whereas mannitol treatment had no effect (p=0.8).

Outcome in patients admitted within 24 hours of stroke onset

In those admitted in the first 24 hours of stroke, case fatality was significantly associated with mannitol treatment at 1 year but not at 30 days (p=0.0388 and p=0.12, respectively). When the effect of mannitol was adjusted for age and the presence of disturbed LOC on admission, mannitol had a significant adverse effect on both 30-day and 1-year survival (p=0.024 and

p=0.0277, respectively). When the SNSS prognostic score was used instead of the LOC, the effect of mannitol became nonsignificant.

Ischaemic stroke patients admitted within 24 hours

When the analysis was further restricted to those 457 patients who had ischaemic stroke and were admitted within 24 hours, mannitol treatment status was not associated with 30-day case fatality (15% in treated and 12.7% in nontreated groups, p=0.51). SNSS prognostic score and case fatality at 30 days and 1 year. The SNSS prognostic score was 16.3±5.6 and 16.8±5.4 in the treated (n=315) and nontreated (n=142) patients, respectively (p=0.21). There was no difference in the frequency of patients with disturbed LOC on admission (p=0.08). Respirator use was similar, whereas nasogastric tube feeding was more frequent in the mannitol group. SNSS long-term score was 26.8±14.7 and 29.9±15.3 in the treated and nontreated groups, respectively (p=0.01).

Case fatality at 1 year was 27.7% in treated and 22.9% in nontreated patients (p=0.28). Although mannitol treatment had the tendency to increase the chances for survival, when treatment effect was adjusted for age, SNSS long-term score, fever in the first 3 days, and aspirin treatment in the acute phase (OR= 1.8693), the confidence intervals were wide and included the possibility of harm.

Cerebral hemorrhage patients admitted within 24 hours

Of the 111 patients with cerebral hemorrhages, 84 were treated and 27 were not treated with mannitol. Although treated patients were older than nontreated patients (65.0±12 and 56.3±12.3 years, respectively; p=0.002), they had similar scores on both the prognostic and long-term items of the SNSS (10.5±6.5 vs 10.1±6.4, p=0.82, and 15.0±14.1 vs 13.6±13.4, p=0.74, in treated and nontreated patients, respectively) and had similar white cell counts and glucose

levels on admission than nontreated patients. Disturbance of the LOC on admission was more frequent in the nontreated group (p=0.02).

Treated and nontreated patients did not differ significantly regarding the frequency of prestroke dependency, chronic obstructive pulmonary disease, malignancy, fever in the first 72 hours, atrial fibrillation, antibiotic use, respirator use, and nasogastric tube feeding. Case fatality was not significantly higher in the treated group at 30 days and 1 year (52% vs 41%, p=0.31, and 62% vs 44%, p=0.12). Although the odds ratios for survival were < 0.6 in all models, suggesting an adverse effect of mannitol treatment, the 95% confidence intervals were wide and included the possibility of a beneficial effect.

5.3. The association of thrombolysis with stroke outcome

5.3.1. Patient characteristics

CTA identified arterial occlusion in 54.7% of the cases. There was no difference in the distribution of risk factors between those with an ASPECT score of ≤7 or >7 on admission or at 24 hours. In those with an ASPECTS ≤7 median NIHSSS was 14 (IQR 11; 17) on admission and 12 (IQR 7; 17) one day later, whereas in those with an ASPECT score of >7, these values were 11 (IQR 8; 17) and 3 (IQR 1; 6) respectively.

5.3.2. Outcome

At 24 hours 6% of our patients became asymptomatic, 34% had NIHSSS ≤7 and 0.8% died. At 3 months 33% of the patients were independent (mRS 0-2), 44% were dependent (mRS 3-5), and 23% were dead. Case fatality at one year was 36%.

5.3.3. Predictors of outcome

Redefining good outcome as mRS 0-1 instead of the predefined mRS 0-2 did not change the conclusions. Those with favorable outcome at 3 months had a median NIHSSS of 8 and 4 on admission and one day later, respectively, whereas those with mRS≥3 at 3 months had a median NIHSSS of 14 both on admission and at 24 hours. Those who survived the first year improved initially (median NIHSSS changed from 10 to 7 in the first 24 hours) whereas in fatal cases there was no improvement during the first day (median NIHSSS was 15 initially and 16 at 24 hours).

The admission ASPECTS was not associated with outcome, but those with a score of ≤7 at 24-hours had significantly worse outcome both at 3 months and at 1 year ($p<0.001$). The outcome was significantly worse in the IA group than those treated IV at 24 hours ($p<0.001$), at 3 months ($p=0.002$) and at one year ($p=0.019$). SICH could be detected six-times more frequently in the IA group ($p<0.001$).

During multivariate analysis ASPECTS on admission, admission glucose level, NIHSSS at 24 hours and treatment modality (IV or IA) retained statistical significance on 3 months outcome. Only 24 hour-NIHSSS predicted case fatality at 1 year ($p<0.001$).

5.3.4. Safety parameters, adverse events

In our cohort case fatality was 0.8% at 24 hours, 23% at 3 months, and 36% at one year. Intracerebral hemorrhage was detected in 59 patients (15.9%). The overall rate of parenchymal hemorrhage was 7% (n=26) and haemorrhagic transformation of the infarction was detected in 33 cases (8.9% of all subjects).

The overall rate of symptomatic intracerebral hemorrhage was 3.5% (n=13) according to both the SITS MOST and the ECASS definitions, and 3.8% using

the RCT NINDS definition. Of the 14 cases with SICH two were bedridden and 12 were dead at 3 months, and all of them were dead at one year.

5. DISCUSSION

5.1. The association of smoking and alcohol consumption with stroke outcome

5.1.1. The relationship of smoking and alcohol consumption with the severity of stroke symptoms

Strokes were not more severe by the NIHSS score in smokers and nonsmokers in our study. Clinical signs at stroke onset, however, were similar in our study in alcohol consumers and nonconsumers. Therefore, we may conclude that at the population level, smoking and heavy alcohol consumption increase stroke-related mortality by increasing the number of cases rather than by increasing stroke severity.

5.1.2. Smoking and stroke severity

We did not find association between smoking and outcome, except for 1-year case fatality in the ischaemic stroke subgroup (18% in smokers and 25% in nonsmokers, p=0.037). The risk factor profile even considering cardiogenic stroke was similar in smokers and nonsmokers according to our studies. The probable explanation for our result could be that smokers in the ischaemic stroke subgroup were over 10 years younger than nonsmokers, which explains this apparent beneficial effect of smoking in univariate analysis. In contrast to the previously mentioned reports, current smoking did not affect functional outcome at 3 months in ischaemic stroke in the study of Aries et al. In the Norfolk study, smoking was a predictor of long-term (mean: 7.5 years) case fatality after stroke,

but long-term case fatality was not associated with any level of alcohol consumption.

The conclusions on the effect of smoking on stroke outcome, therefore, are conflicting, and the results mostly depend on the length of follow-up and on the statistical method used in individual studies. The harmful effect of smoking becomes more obvious after longer follow-ups when other adverse effects of continuing smoking like bronchial cancer and pneumonia will manifest and increase case fatality. As smoking is more prevalent in younger stroke patients, the illusion of a beneficial effect of smoking suggested by univariate statistical tests in the relatively short-term outcome studies disappears in multivariate analyses, when age is considered as a confounder.

5.1.3. Chronic alcohol consumption and stroke outcome

We found no difference between alcohol consumers and nonconsumers in 30-day and 1-year case fatality in all stroke patients and in ischaemic stroke patients. In the ICH subgroup, we found a tendency for higher case fatality in alcohol consumers at 30 days and at 1 year as well; however, the difference was not statistically significant in univariate (chi square) and in multivariate testing (logistic regression).

Our study has some limitations. Neither alcohol consumption nor smoking was recorded by patient diary, but we relied on the information we got from patients or their relatives. It is difficult to obtain reliable data on alcohol consumption and smoking. We could not obtain data for alcohol consumption in 20.5% and for smoking in 10.4% of cases. On one hand, this might have resulted in some selection bias against more severe strokes: both stroke severity and outcome were more severe in those without reliable data on smoking and alcohol habits. On the other hand, our conclusions on the effect of smoking and

alcohol consumption may be more reliable as those with missing information were excluded from data analysis.

The number of those with ischaemic strokes are large enough for reliable conclusions; however, the number of those with ICH (n=128) are relatively low, so nonsignificant tendencies for ICH shall have to be further checked in a larger number of ICH patients. The strength of this study is the sufficient followup rate, which was possible in over 96% of cases.

Overall, our data suggest that despite being risk factors, prestroke smoking and alcohol consumption status do not have a significant influence on initial stroke severity and on short- and long-term outcome after stroke, especially when confounding factors, like age, are considered in multivariate analyses. These findings should be confirmed by further studies and analyses of larger databases. Our data suggest that in populations with a considerable proportion of smokers and alcohol consumers -like those in Central-Eastern Europe - smoking and heavy alcohol consumption increase stroke-related mortality rather by increasing stroke incidence than by increasing stroke severity.

5.2. The association of mannitol treatment with stroke outcome

Although the results of observational studies on treatment effects and on case fatality should be very cautiously interpreted because of the potential of large biases, the results were surprising to us. In contrast to the expected favorable effect of mannitol, we could not find any association between mannitol use and better prognosis at 30 days and 1 year after stroke. Depending on the factors included in the logistic regression models, mannitol either did not have a significant effect on case fatality or was associated with an adverse outcome. Of the neurologic signs on admission, in the analysis we first used only the LOC, because among the clinical signs, this was found to be the most significant prognostic factor in ischaemic and haemorrhagic strokes.

When disturbed LOC and age were used as confounding factors in the model, mannitol had a significant negative effect. However, when LOC was changed to a more complex, quasi-continuous index of neurologic damage, ie, the prognostic score of the SNSS including LOC, eye movements, and severity of paresis of the affected upper and lower extremities, the effect of mannitol became nonsignificant. When the analysis was restricted to those who were admitted within 24 hours, the findings were similar.

The fact that mannitol use was associated with higher short-term and long-term case fatality in the total group might be partly or totally explained by the differences between treated and nontreated patients in prognostic factors. The absolute difference in SNSS prognostic scores between treated and nontreated patients was small, though statistically significant, in the total group, but was not significant in the subgroup comparisons. Most prognostic and confounding factors did not differ significantly between treated and nontreated patients admitted within 24 hours with ischaemic stroke, and several factors were even more favorable for treated patients in the haemorrhagic subgroup.

This analysis has several limitations. 1. This is a prospective, observational study and not a randomized, controlled trial; therefore, selection bias could have affected the results. 2. Since this was not a randomized controlled trial unknown factors- not included in the study- could have influenced outcome as well. 3. Computed tomography (CT) was performed in only 73% of the patients, and in 10% of the patients, no lesion was detected. In these patients, no repeated scans were performed. Therefore, it was not possible to perform an analysis by the volume of the lesions. It has been reported that although a visible infarct on the CT scan is associated with adverse prognosis, at least 30% of patients with ischaemic strokes have normal CT scans. 4. There were missing data for some of the patients for several parameters; e.g., white cell count was present for > 95% of patients, but data for glucose level on admission were missing in close to

20% of patients. 5. Mannitol was administered according to the discretion of the treating neurologist, and the dose of mannitol used and the duration of treatment varied.

We attempted to analyze the effect of mannitol on case fatality by considering as many confounding factors as possible. It was not possible to enter into the model all factors that possibly influence short- and long-term survival. Depending on the number and type of prognostic factors used in the multivariate analysis, mannitol had either a nonsignificant or an adverse effect. This observational study does not prove that mannitol is harmful if given for acute stroke, but it raises concerns and emphasizes the need for properly designed, randomized, clinical trials to decide whether the practice of routine mannitol use in patients with acute stroke is justified, should be restricted to subgroups, or should be stopped altogether.

5.3. Factors that may have an effect on the outcome of stroke after thrombolysis

5.3.1. Baseline characteristics

Mean age of our rtPA treated patients is similar to that in France, Germany, Denmark and Switzerland, but lower than in Greece, Italy or Sweden. Hypertension and prestroke cardiac failure were more frequent in the Hungarian sample compared to the SITS MOST study. The rate of hypertension is much higher in Hungary than in most European countries, but similar to values reported from the USA and Canada. Atrial fibrillation (16.8% in the Debrecen region) is underrepresented compared to values in France (24.7%) and Sweden (33.3%), but similar to data from Germany (14.1%). The occurrence of diabetes mellitus was similar to that in the ECASS 3 population (14.8 vs. 16.6%). The

proportion of smokers in our cohort is 25%, lower than reported in ECASS 3 (30.6-28.8%), or in SITS MOST (43.2%).

5.3.2. Logistic issues (onset to door time, door to needle time, onset to treatment time)

The logistic parameters of our center were similar to international data. Guidelines recommend since 2013 <60 minutes door to needle time. According to the experience in Finland, with optimal organization of prehospital and in-hospital stroke and thrombolysis management much shorter times can be achieved.

5.3.3. Imaging

CT angiography is performed routinely in our center leading to discovering vessel occlusion in 54.7% of the cases. In the NINDS study CTA was not performed, the rate of large vessel occlusion was estimated as 35-39% based on clinical criteria. The higher rate of large vessel occlusion could be one of the reasons for the worse long term outcome in our patients.

Most of our patients had an ASPECT score of 10 before thrombolysis and even after 24 hours an ASPECT score of 10 was found in every seventh patient on the repeated scan. The admission ASPECTS (>7 vs≤7) was not associated with long term outcome in univariate analysis. The ASPECTS at 24 hours reflecting permanent damage had a significant effect on outcome both at 3 months and at one year. With multivariate analysis ASPECTS on admission was an independent predictor at 3 months. We found a non-significant unfavorable association of hyperglycemia (>10 mmol/L serum glucose level) with worse ASPECT score at 24 hours.

5.3.4. Short-term outcome

Initial stroke severity in our patients (median NIHSSS: 12) was within the range of published data of the ECASS 3 (median: 9-10), the SITS MOST (median: 12), and the NINDS study (median: 14). The thrombolysis was not only effective, but safe as well. We detected SICH in 3.8% (i.e. 23.7% of all intracerebral hemorrhages became symptomatic) after treatment, similar to the SICH ratios in ECASS 3 (2.4% and 27%), and lower than reported in the ECASS II of 6.2%, or in the NINDS study of 6-7%. A Canadian thrombolysis registry found a 4.6% SICH rate. In the NINDS and ECASS II trials case fatality in SICH cases was 50-75% in different subgroups at 3 months and 61% at one year. Case fatality was higher among our patients with SICH: 86% at 3 months, and 100% at one year. It should be noted that in the NINDS and ECASS II patients were treated within 180 minutes and in a frame of a clinical trial. A recent meta-analysis also reported that those patients benefit from the rtPA treatment who do not develop SICH and survive the acute phase.

5.3.5. Long-term outcome

In our database 1 out of 3 patients had good recovery at 3 months, and 2 out of 3 were alive at one year. We found that the presence of hyperacute signs of ischemia by neuroimaging, admission glucose level, and the 24 hour-NIHSSS on long-term outcome, and IA treatment were independent predictors of disability at 3 months. A recent trial in patients with acute ischaemic stroke indicated that IA therapy is not superior to standard treatment. As our study was not randomized, the effect of IV and IA treatment cannot be compared, and the effect of unknown or unconsidered confounders might explain the worse outcome after IA rtPA treatment in our patients.

Compared to international data survival rate was lower (64.5% at one year) in our database. The most probable explanations of the worse outcome in our

cohort therefore are the longer time window (4.5 vs. 3 hours) and the higher rate of large vessel occlusion compared to the NINDS study. Further, our study was a real world observational study, including such patients as well who should have been excluded from clinical trials.

Our study has several limitations. The advantage is prospective data collection and detailed information on all subjects. The number of patients involved however is limited and the study is possibly underpowered to identify all clinically important predictors of long term outcome after thrombolysis. This may explain why important clinical features like age, on-admission NIHSSS, the presence of large vessel occlusion, prestroke heart failure, atrial fibrillation and lipid-lowering therapy found to be significantly associated with long-term outcome in univariate testing, lost their statistical significance in multivariate analyses.

However, even with this relatively small number of cases, we could identify the strongest predictors of long term outcome, i.e. severity of stroke signs 24 hours after onset, serum glucose on admission, findings on initial neuroimaging and treatment modality.

Compared to international data thrombolysis was similarly effective and safe in our patients in the short term, but the outcome at 3 months and at one year were worse. A higher proportion of large vessel occlusion, longer time window, a higher rate of some risk factors and concomitant diseases may be partly responsible for the worse long term outcome in our patients. The need for a more efficient care to prevent complications after the acute phase of stroke and the importance of early rehabilitation should also be emphasized.

NEW ESTABLISHMENTS

I. Despite being risk factors, pre-stroke smoking and chronic alcohol consumption status do not have significant influence on initial stroke severity and on short- and long- term outcome after stroke, especially when confounding factors, like age are considered in multivariate analyses. Our data suggest that in populations with a considerable proportion of smokers and alcohol consumers – like those in Central-Eastern Europe - smoking and heavy alcohol consumption may increase stroke related mortality rather by increasing stroke incidence than by increasing stroke severity.

II. In multivariate analysis, mannitol had either non-significant or unfavorable association with stroke outcome. This result does not prove that mannitol is harmful in acute stroke, but raises concerns and emphasizes the need for properly designed, randomized, clinical trials to decide whether the practice of routine mannitol use in patients with acute stroke is justified, should be restricted to subgroups, or should be stopped altogether.

III. We established the prospective Debrecen Thrombolysis Database. Compared to international data thrombolysis was similarly effective and safe in our patients in the short term, but the outcome at 3 months and at one year were worse. At 3 months one third of the patients were independent and at one year 2 out of 3 patients were alive. Significant independent predictors of disability at 3 months were 24 hour-NIHSSS, admission ASPECTS, admission glucose level, and treatment modality, whereas at one year after stroke only the 24 hour-NIHSSS was a significant predictor of case fatality.

Register Number: DEENKÉTIK/38/2014.
Item Number:
Subject: Ph.D. List of Publications

Candidate: Klára Fekete
Neptun ID: XKJO4S
Doctoral School: Doctoral School of Clinical Medicine
Mmt ID: 10038837

List of publications related to the dissertation

1. **Fekete, K.**, Szalmári, S., Szőcs, I., Szekeres, C., Szász, J., Mihálka, L., Smolanka, V., Kardos, L., Csiba, L., Bereczki, D.: Prestroke Alcohol Consumption and Smoking Are Not Associated with Stroke Severity, Disability at Discharge, and Case Fatality.
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Total IF of journals (all publications): 39.428

Total IF of journals (publications related to the dissertation): 7.217

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

27 February, 2014

Publication sent to Journal of Stroke and Cerebrovascular Diseases in review:

Fekete K, Márton S, Tóth J, Csiba L, Fekete I, Bereczki D. Predictors of long-term outcome after rtPA treatment in the Eastern Hungarian Thrombolysis Database. (J Stroke Cerebrovasc Dis, under review)