RESEARCH ARTICLE

GFAP and S100B Protein are Associated With Discharged NIHSS of Anterior Circulation Ischemic Stroke

Yenny Surjawan[1,2*], Suryani As'ad[1], Teguh A.S. Ranakusuma[3], Andi Wijaya[1,2]

[1]Post Graduate Program in Clinical Biochemistry, Hasanuddin University, Jl. Perintis Kemerdekaan Km.10. Makassar, Indonesia
[2]Prodia Clinical Laboratory, National Refrence Lab., Prodia Tower 2nd fl., Jl. Kramat Raya No. 150, Jakarta, Indonesia
[3]Faculty of Medicine, University of Indonesia, Jl. Salemba Raya No.6, Jakarta, Indonesia
*Correspondence: e-mail: yensurjawan@yahoo.com

Abstract

BACKGROUND: Patient with larger ischemic lesion will suffer more severe neurological deficit. The utility of MRI for lesion size measurement is still limited, therefore additional approach was pursued through examination of markers released by damaged brain cell, GFAP and S100B protein. The aim of this study is to know whether both markers are associated with the neurological deficit of anterior circulation ischemic stroke.

METHODS: This observational prospective study enrolled 74 patients with anterior circulation ischemic stroke diagnosis. GFAP and S100B protein were measured with ELISA using blood collected at 48 to 72 hours after onset. The neurological deficit was assessed with NIHSS at discharge.

RESULTS: There was a significant association between GFAP level and discharged NIHSS (p = 0.008) with 100 % sensitivity and 100 % negative predictive value. S100B protein also showed a significant correlation with discharged NIHSS (r = 0.488; p = 0.000) and this correlation could be described with an equation (OR = 1.009; 95 % CI 1.0003 - 1.0188; p = 0.044). S100B protein at 78.3215 ng/l would give true prediction as 73.9 % (95 % CI 62.7 % – 85.2 %, p = 0.001).

Abstrak

LATAR BELAKANG: Pasien stroke iskemik dengan lesi yang lebih luas biasanya akan mengalami defisit neurologis yang lebih berat. Pengukuran luas lesi dengan MRI masih terbatas penggunaannya, oleh karena itu diupayakan suatu pendekatan lain melalui pemeriksaan protein S100B dan GFAP yang merupakan petanda kerusakan sel otak. Tujuan penelitian ini untuk mengetahui apakah kedua petanda tersebut berhubungan dengan beratnya defisit neurologis pada pasien stroke iskemik sirkulasi anterior.

METODA: Studi observasional dengan pendekatan prospektif ini dilakukan terhadap 74 pasien dengan diagnosis sebagai stroke iskemik sirkulasi anterior. GFAP dan protein S100B diperiksa dengan metode ELISA menggunakan darah yang diambil pada jam ke 48–72 jam setelah onset. Beratnya defisit neurologis dinilai melalui pemeriksaan skor NIHSS saat pulang.

HASIL: GFAP menunjukkan hubungan yang bermakna dengan NIHSS saat pulang (p = 0.008) dengan nilai sensitivitas sebesar 100 % dan nilai prediksi negatif sebesar 100 %. Protein S100B juga menunjukkan korelasi yang bermakna dengan NIHSS saat pulang (r = 0.488; p = 0.000) dan korelasi ini dapat digambarkan melalui suatu

CONCLUSION: GFAP and S100B protein that were measured at 48 to 72 hours after onset were significantly associated with NIHSS at discharge.

KEYWORDS: GFAP, S100B protein, discharged NIHSS, ischemic stroke

Indones Biomed J 2012; 4 (2): 112–117

persamaan (OR = 1.009; 95 % CI 1.003 – 1.0188; p = 0.044). Pada kadar 78.3215 ng/l, protein S100B dapat memprediksi 73.9 % kasus secara benar (95 % CI 62.7 – 85.2; p = 0.001).

KESIMPULAN: GFAP dan protein S100B yang diukur pada jam ke 48–72 jam setelah onset menunjukkan hubungan bermakna dengan NIHSS saat pulang.

KATA KUNCI: GFAP, S100B protein, NIHSS saat pulang, stroke iskemik

Indones Biomed J 2012; 4 (2): 112–117

Introduction

Based on Indonesian Health Profile 2008, stroke still ranks as the first cause of death in Indonesian (1). Stroke patients who survived generally suffered a disability. Most of post stroke patients will have sequelae with varying degrees of severity (2).

The severity of stroke is commonly determined by the location and the size of the ischemic lesion (2,3). In several studies, larger lesion which is associated with worse outcome was reported to have a higher concentration of glial fibrillary acidic protein (GFAP) and S100B protein (4,5,6,7). The aim of this study is to know whether GFAP and S100B protein are associated with the discharged National Institute of Health Stroke Scale (NIHSS) after anterior circulation ischemic stroke.

Methods

It is an observational study with a prospective design performed on 74 anterior circulation ischemic stroke patients who came to the hospital not more than 72 hours after the onset. Subjects were enrolled from several hospitals in Jakarta and Makassar since June 2011 until May 2012. The diagnosis of anterior circulation ischemic stroke was made by neurologist based on the data of anamnesis, physical examination, and proved with CT or MRI. Either single or multiple infarct was included in this study. This study was approved by the Health Research Ethics Committee, Medical Faculty of Hasanuddin University, Makassar, Indonesia.

Stroke subjects who had a first attack and received a standard medical care were included in this study. Patients who had liver dysfunction (female: AST > 54 U/L, ALT > 68 U/L; male: AST > 66 U/L, ALT > 100 U/L), serum creatinine > 1.6 mg/dl [141.44 µmol/L], random blood glucose < 55 mg/dl [3.0525 mmol/L], systolic blood pressure < 100 mmHg or diastolic blood pressure < 70 mmHg, and history of cancer, acute myocardial infarction, or congestive heart disease within the last 3 months were excluded from this study.

Blood was collected between 48 to 72 hours after the onset of stroke. Serum for GFAP and S100B protein measurement were kept at < 20°C until all samples were ready to be analyzed and the diagnosis of anterior circulation ischemic stroke was confirmed. The concentration of GFAP was measured with ELISA method using Human GFAP ELISA RD reagent from *Biovendor* (Cat. RD 192072200R) and the result was reported in ng/ml. S100B protein was measured with ELISA method using CanAg S100BB EIA from Fujirebio (Cat. 708-10) and the result was reported in ng/l. The assays were performed at Research Department of Prodia Clinical Laboratory, Jakarta, Indonesia.

Stroke outcome was recorded when the patient was discharged from hospital. Patients who survived were assessed for their NIHSS by a trained assistant or neurologist. The results were noted with the range from 0 as the minimal score to 42 as the maximal score. The NIHSS was classified as mild, moderate and severe

if the score was less than 4, 4 to 15, and more than 15, respectively. Non survive patients were considered to have the maximum score of NIHSS.

Data was analysed using SPSS 13.0 statistical analysis software for Windows (SPSS Inc., Chicago, IL, USA). The significant level of this analysis was set at 5%.

Table 1. Subject's baseline characteristics (N = 74)

Variable	N (%)
Onset (hours)	
<24	68 (91.9)
>24	6 (8.1)
Age (years old)	
33– 52	23 (31.1)
53 – 63	32 (43.2)
64 – 79	19 (25.7)
Gender	
Male	45 (60.8)
Female	29 (39.2)
Admission NIHSS	
Mild (0 - 3)	15 (20.3)
Moderate (4 – 15)	58 (78.4)
Severe (16 – 42)	1 (1.4)
Discharged NIHSS*	
Mild (0 – 3)	30 (40.5)
Moderate (4 – 15)	42 (56.8)
Severe (16 – 42)	2 (2.7)
NIHSS progression	
Better	49 (66.2)
Stable	21 (28.4)
Worse	4 (5.4)
Status at discharge	
Survive	72 (97.3)
Non survive	2 (2.7)
GFAP level (ng/ml)	
Low (<0.2500)	67 (90.5)
High (>0.2500)	7 (9.5)
S100B protein concentration (ng/l)*	
Q1: 51.58 (33.02 – 57.79)	20 (27.0)
Q2: 67.11 (61.52 -77.70)	16 (21.6)
Q3: 90.18 (78.95 – 110.23)	20 (27.0)
Q4: 265.27 (148.04 – 2553.08)	18 (24.3)

*Data distribution was not normal (Kolmogorov Smirnov test, $p < 0.05$)

Results

Data analysis was performed on 74 subjects with anterior circulation ischemic stroke diagnosis. The baseline characteristics of these subjects were shown in Table 1. Most of these subjects were admitted to hospital within 24 hours after onset and had moderate admission NIHSS (78.4%). At discharge from hospital there were 4 subjects (5.4%) with severe NIHSS and 2 of them died, while almost all of the remaining subjects showed improvement in their NIHSS (66.2%).

The concentration of S100B protein ranged from 33.02 to 2553.08 ng/l. The median and the minimal to maximal concentration of each quartiles of S100B protein were shown in Table 1. The level of GFAP were classified as low if the concentration of GFAP was less than the lowest value of calibrator (< 0.2500 ng/ml) and high if the concentration was greater or equal to the lowest value of calibrator (≥ 0.2500 ng/ml).

There was a significant difference in the proportion of subjects with low GFAP and high GFAP in either non severe or severe group of NIHSS at discharge (Fisher's exact test, $p = 0.008$) (Table 2). The assay of GFAP showed a sensitivity of 100% (95% CI 15.8–100%) and specificity of 93.1% (95% CI 84.5–97.7%) with positive predictive value of 28.6% (95% CI 3.7–71.0%) and negative predictive value of 100 % (95 % CI 94.6–100).

Table 2. Crosstab between GFAP level and status of NIHSS at discharge

	Severe discharged NIHSS		
	Yes	No	Total
High GFAP	2	5	7
Low GFAP	0	67	67
Total	2	72	

The concentration of S100B protein was significantly different among NIHSS groups (Kruskal Wallis test, p = 0.001). Further post hoc analysis using Mann Whitney test revealed a significant difference of S100B protein concentration between subjects with mild and moderate discharged NIHSS (p = 0.001), and also between mild and severe discharged NIHSS (p = 0.024). The median concentration of S100B protein in the severe group was higher than the median concentration in the moderate group, but the difference was not significant (p = 0.055) (Figure 1).

Spearman correlation test showed that there was a significant correlation between S100B protein and discharged NIHSS (r = 0.488; p = 0.000). The probability to get a non mild discharged NIHSS by S100B protein concentration might be predicted from an equation (p = $1 / \{1 + 2.7^{6.30 - 0.009 \times \text{S100B protein}}\}$) with OR = 1.009; 95% CI 1.0003 - 1.0188 (p = 0.044).

The discrimination ability of this equation was checked with receiver operating characteristics (ROC) (Figure 2) that produced an area under curve (AUC) of 73.9% (p = 0.001, 95% CI 62.7%–85.2%). The cut-off value of S100B protein at 78.3215 ng/l produced a sensitivity of 66% and specificity of 70%, with positive predictive value and negative predictive value of 76% and 58%, respectively.

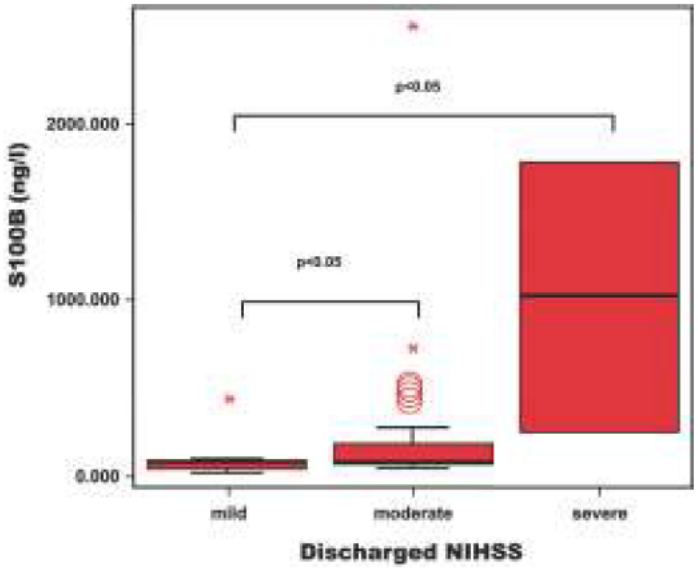

Figure 1. S100B protein concentration in mild, moderate, and severe discharged NIHSS.

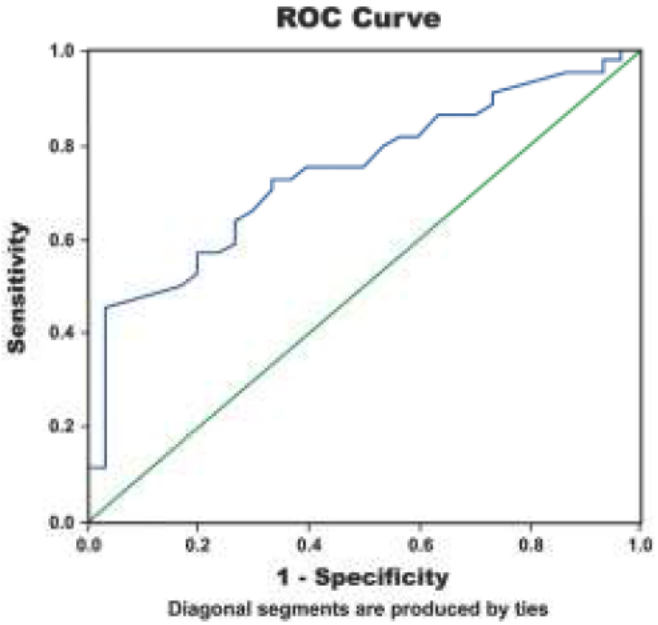

Figure 2. ROC curve of S100B protein to discriminate discharged NIHSS.

Discussion

In ischemic stroke, the cerebral blood flow is disturbed because of the obstruction of cerebral blood vessel. Energy that is required for normal brain metabolism is reduced and the ischemic brain cells are unable to control the passage of ions through cell membranes. The ions imbalance will trigger a sequential biochemical reaction (ischemic cascade) until finally the brain cells disintegrate and die (8).

The disintegration of brain cell membranes will cause the GFAP known as the cytoskeleton of astrocyte and the S100B protein located in the cytoplasmic of astrocytes, to be released into the cerebrospinal fluid (9,10). The markers of the brain damage will leak to systemic circulation as the blood brain barrier is also disrupted (11).

A previous study by Herrmann *et al.*, showed that GFAP level had a correlation with both lesion size ($r = 0.955$, $p < 0.0001$) and NIHSS ($r = 0.717$, $p = 0.0003$) (12). In our study, GFAP level showed a significant association with discharged NIHSS ($p = 0.008$). All patients with severe discharged NIHSS showed high GFAP level. No patients with low GFAP level had severe discharged NIHSS. Based on this high sensitivity and negative predictive value, the low level of GFAP might be considered as an indicator to exclude the possibility to get a severe NIHSS at discharge.

There were only 2 of 7 subjects with high level of GFAP (0.2870 and 13.7590 ng/ml) who were discharged with severe NIHSS, but both of these patients were die. High GFAP might be associated with mortality.

S100B protein also showed a positive significant correlation with discharged NIHSS ($r = 0.488$; $p = 0.000$). Patients with higher concentration of S100B protein would be discharged from hospital with higher NIHSS. This was in accordance with the result previous study that showed a correlation of S100B protein concentration with lesion size ($r = 0.957$, $p < 0.0001$) and neurological deficit measured by NIHSS. The strongest correlation of S100B protein and NIHSS was found when the patient was discharged from hospital ($r = 0.821$, $p = 0.0002$) (12).

A significant difference of S100B protein concentration was found between mild and non mild (moderate and severe) discharged NIHSS ($p < 0.05$). The probability to get a non mild discharged NIHSS could be predicted by S100B. Alhough the OR in this study was only 1.009, it could give a significant estimation of how big the risk and probability to get a not mild discharged NIHSS by S100B concentration as illustrated in Table 4. This due to the S100B protein concentration in this study had a quite large range (33.02–2553.08 ng/l). The true prediction of the equation was 73.9%. The patient would have a non mild discharged NIHSS if they had S100B protein concentration greater than or equal to 78.3215 ng/l ($p = 0.001$, 95% CI 62.7%–85.2%). This assay showed a sensitivity of 66% and specificity of 70% with 76% positive predictive value and 58% negative predictive value.

Table 4. The risk and probability to get a non mild discharged NIHSS based on S100B protein

S100B protein (ng/l)	Risk	Probability (%)
1	1.009	0.18
50	1.57	0.28
100	2.46	0.45
500	90.2	14.19
1000	8103.08	93.70
1500	729416.37	99.92
2000	65659969.14	100
2500	5910522063.02	100

In addition to lesion size, the severity of neurological deficit is also influenced by the location of ischemic stroke (8). Our study tried to limit this influence by selecting subjects whose lesion were in anterior circulation area. But, this was still a pitfall of this study because the large lesion in frontal will do much better than the very small lesion at internal capsul, whereas both are distribuded by anterior circulation (8).

The association of GFAP and S100B protein with discharged NIHSS in this study needs to be validated before it can be utilized. More significant prediction might be obtained if the result of S100B protein and GFAP is combined with other known clinical variables that influence the outcome of stroke (multivariable predictor) (4,13).

Acknowledgement:

We thank Prodia Education and Research Institute for funding this research.

References:

1. Indonesian Health Profile 2008. Jakarta: Ministry of Health, Republic of Indonesia; 2010.
2. Parham, S. New biomarkers could deliver a strike at stroke: Feature Story. CAP Today. 2004 November.
3. Whiteley W, Tseng MC, Sandercock P. Blood biomarker in the diagnosis of ischemic stroke: A systematic review. Stroke 2008;39:2902-9.
4. Whiteley W, Wei LC, Sengupta A, Sandercock P. Blood markers for the prognosis of ischemic stroke: a systematic review. Stroke 2009;40:e380-9.
5. Buttner T, Weyers S, Postert T, Sprengelmeyer R, Kuhn W. S-100 protein: serum marker of focal brain damage after ischemic territorial MCA infarction. Stroke 1997;28:1961-5.
6. Missler U, Wiesmann M, Wittmann G, Magerkurth O, Hagenstrom H. Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results. Clin Chem 1999;45:138-41.
7. Wunderlich, MT, Ebert AD, Kratz T, Goertler M, Jost S, Herrmann M. Early neurobehavioral outcome after stroke is related to release of neurobiochemical markers of brain damage. Stroke 1999;30:1190-5.
8. Caplan LR. Caplan's Stroke: A Clinical Approach. 4th ed. Philadelphia: Saunders; 2009.
9. Michetti F, Gazzolo D. S100B Protein in biological fluids: a tool for perinatal medicine. Clin Chem 2002;48:2097-104.
10. Herrmann M, Vos P, Wunderlich MT, de Bruijn CHMM, Lamers KJB. Release of glial tissue-specific proteins after acute stroke: a comparative analysis of serum concentration of protein S-100B and glial fibrillary acidic protein. Stroke 2000;31:2670-7.
11. Hill MD. Diagnostic biomarkers for stroke: a stroke neurologist's perspective. Clin Chem 2005;51:2001-2.
12. Foerch C, Curdt I, Yan B, Dvorak F, Hermans M, Berkefeld J, *et al.* Serum glial fibrillary acidic protein as a biomarker for intracerebral haemorrhage in patients with acute stroke. J. Neurol. Neurosurg. Psychiatry 2006;77:181-4.
13. Maas MB, Furie KL. Molecular biomarkers in stroke diagnosis and prognosis. Biomark Med 2009;3:363-83.