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RESEARCH ARTICLE

S100B and brain natriuretic peptide predict functional neurological outcome after intracerebral haemorrhage

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Abstract

Objective: To determine the predictive value of S100b and brain natriuretic peptide (BNP) in order to determine accurately and quickly a discharge prognosis after primary supratentorial intracerebral haemorrhage (ICH).

Methods: After IRB approval and informed consent, blood samples were obtained and analysed from 28 adult patients consecutively admitted to the neuroscience intensive care unit with computed tomography-proven supratentorial ICH from June 2003 and December 2004 within the first 24 h after symptom onset for S100b and BNP. Functional outcomes on discharge were dichotomized to favourable (mRS <3) or unfavourable.

Results: BNP (a neurohormone) and S100b (a marker of glial activation) were found to be independently highly predictive of functional neurological outcome at the time of discharge as measured by the modified Rankin Score (BNP: $p<0.01$, $r=0.46$; S100b: $p<0.01$, $r=0.42$) and the Barthel Index (BNP: $p<0.01$, $r=0.54$; s100b: $p<0.01$, $r=0.50$). Although inclusion of either biomarker produced additive value when included with traditional clinical prognostic variables, such as the ICH score (Barthel index: $p<0.01$, $r=0.66$; mRS: $p<0.01$, $r=0.96$), little predictive power is added with inclusion of both biomarkers in a regression model for neurological outcome. *Conclusions:* Serum S100b and BNP levels in the first 24 h after injury accurately predict neurological function at discharge after supratentorial ICH.

Keywords: *Intracerebral haemorrhage; prognosis; s100 proteins; brain natriuretic peptide; intensive care; neurological outcome*

Introduction

Primary supratentorial intracerebral haemorrhage (ICH) is a relatively common and devastating disease with little improvement in functional neurological outcome over the past decade despite advances in medical technology (Broderick et al. 2007). While acute diagnosis is now relatively straightforward, since the advent of computed tomography (CT) scanning, the ultimate prognosis remains difficult to predict early in the disease course, especially in light of the decision of many families to 'withdraw care' on patients deemed unlikely to have favourable long-term functional outcome by their physicians. This uncertainty has resulted in a wide spectrum of patient outcomes, from complete rehabilitation to persistent vegetative state, underscoring the need for adjunctive prognostic tools to guide initial management decisions in the setting of acute ICH.

Traditionally, in the acute setting, prognosis following ICH has been guided by several variables, including age, haematoma location, size and ventricular extension (Garibi et al. 2002, Juvela 1995). However, these traditional prognostic variables remain imperfect, and are often not adequate to provide a realistic assessment of likely outcome in an individual patient. Although direct neuronal injury from the mass effect of the haematoma plays an important role in determining outcome, advances in the critical care arena have resulted in some patients

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surviving this initial insult only to deteriorate due secondary injury from cerebral oedema. Consequently, it is increasingly recognized that neuroinflammation plays an important role in mediating this cerebral oedema, secondary neuronal injury and, ultimately, functional outcome (James et al. 2008). Thus, it is feasible that biochemical markers of neuronal injury and inflammation might provide adjunctive prognostic information, as well as a surrogate measure of neuropathophysiology to assess the effect of potential therapeutic interventions.

The role of biomarkers has previously been described in other mechanisms of acute brain injury, including ischaemic stroke (Lynch et al. 2004, Nakagawa et al. 2005), traumatic brain injury (Sviri et al. 2006) and subarachnoid haemorrhage (McGirt et al. 2004, Stranjalis et al. 2007). In particular, markers reflective of astrocytic activation, such as S100B, have a relatively large body of evidence demonstrating elevation after acute ischaemic stroke (Lynch et al. 2004, Mizukoshi et al. 2005, Reynolds et al. 2003) and subarachnoid haemorrhage (Lynch et al. 2005). Moreover, S100B has shown some promise as a surrogate marker for therapeutic intervention in ischaemic stroke (Elting et al. 2002) and a predictor of haemorrhagic conversion and outcome (Foerch et al. 2006, Foerch et al. 2007, Jonsson et al. 2001, Kokocinska et al. 2007). Furthermore, a recent examination of S100B levels after subarachnoid haemorrhage revealed a correlation with long-term functional outcome and also disclosed the complex nature of the clinical use of serological markers, in that their utility for prognosis can be limited in clinically relevant timeframes (Sanchez-Pena et al. 2008). Finally, S100B is one of a small number of serological proteins previously demonstrated to correlate with functional outcome after ICH (Delgado et al. 2006).

Brain natriuretic peptide (BNP), although not previously evaluated specifically after ICH, has historically been assessed in the setting of heart failure (Valli et al. 1999) and recent data have demonstrated elevated BNP levels after acute brain injuries, such as ischaemic stroke (Koenig et al. 2007, Nakagawa et al. 2005), traumatic brain injury (Sviri et al. 2006) and subarachnoid haemorrhage (Fukui et al. 2004, McGirt et al. 2004). To date, the relevance of BNP in the setting of acute ICH is poorly understood. However, there is emerging preclinical evidence to suggest that BNP may play an adaptive role in recovery from acute brain injury, possibly through augmentation of cerebral blood flow (Anon 2008).

In the current study, we evaluate the hypothesis that these two biomarkers, S100B and BNP, both shown previously to provide diagnostic and prognostic significance in other mechanisms of acute brain injury, would provide greater benefit for prognosis after supratentorial primary ICH than traditional clinical and radiographic criteria, or either biomarker alone.

Methods

Patients

Following approval from the Duke University Medical Center Institutional Review Board, patients were enrolled after written, informed consent was obtained from the study participants or their legal designates. The primary endpoint in this study was functional neurological outcome at the time of hospital discharge as assessed by the Barthel Index (BI) and modified Rankin Score (mRS). Supratentorial ICH was confirmed by CT imaging prior to enrolment. Exclusion criteria included age less than 18 years, confirmed pregnancy, known or suspected brain tumour, known or suspected central nervous system (CNS) vascular malformation, presence of subarachnoid blood, massive head trauma, time of presentation greater than 24 h after symptom onset, or multiple organ dysfunction at the time of admission.

Blood samples were taken from 28 consecutive patients admitted to the Neuroscience Intensive Care Unit (NICU) between 1 January 2000 and 31 December 2003, with CT-proven supratentorial ICH during multiple timeframes from symptom onset (0–6 h, 6–12 h and 12–24 h). The highest serum value recorded in the first 24 h after symptom onset was used for the analysis and comparisons. Symptom onset was defined as the last known time of baseline neurological function. Upon entry into the study, demographics were recorded and the Glasgow Coma Score (GCS), NIH Stroke Scale (NIHSS) and ICH Score (Hemphill et al. 2001) were tabulated. Briefly, the ICH Score is a 6-point scale validated to stratify risk after primary ICH in humans and consists of initial GCS, haematoma volume and location, presence of intraventricular blood and age. During the first 36 h after admission, CT scans were obtained and evaluated for haemorrhage volume (cm^3), midline shift (MLS, mm), and presence of intraventricular haemorrhage (IVH); additionally the latency from ictus to the study scan was recorded. Upon discharge from the hospital, mRS, BI, hospital length of stay (LOS) and NICU LOS were recorded by a single observer blinded to the biomarker data.

Immunoassay procedure

Blood drawn from each patient was centrifuged (10 000*g*), and the resulting supernatant was immediately frozen at 70°C until analysis was completed. Measurements of biochemical markers involved in the inflammatory cascade were performed by Biosite, Inc. (San Diego, CA, USA). All immunoassays were forward immunometric (sandwich) assays performed in 384-well microtitre plates using a Tecan Genesis RSP 200/8 Workstation (Tecan US, Durham, NC, USA). Each plasma sample was assayed in duplicate. The biotinylated antibody

was added to neutravidin-coated 384-well black plates (Pierce Chemical Co., Rockford, IL, USA) and incubated at room temperature for 1 h. The plate was washed, and then plasma samples (10 µl) were aliquoted into the wells. After incubation for 1.25 h, the plate was washed again, and the alkaline phosphatase-conjugated antibody was added. After an additional incubation for 1.25 h, the plate was washed a third time, and AtttoPhos® substrate (JBL Scientific, San Luis Obispo, CA, USA) was added to measure the amount of alkaline phosphatase-conjugated antibody bound in each well. The plates were read by a fluorometer with an excitation wavelength of 430 nm and an emission wavelength of 570 nm. Each well was read six times at 114-s intervals, and a rate of fluorescence generation was calculated. Calibrators were prepared gravimetrically in pooled plasma from healthy donors. One tube in each set of calibrators included neutralizing antibody for correction of endogenous antigen present in the plasma pool. Calibration curves were eight points run in duplicate in columns 1 and 2 and repeated in columns 23 and 24 of the assay plate. The calibration curve was calculated using a four-parameter logistic fit (Reynolds et al. 2003).

Imaging

All patients enrolled into the study were diagnosed with CT-proven supratentorial ICH prior to blood sampling. Because re-bleeding and initial cerebral oedema formation occur within the first 24 h after ictus, the imaging scan used for the study protocol was the next subsequent scan that occurred within 36 h after admission. A blinded neuroradiologist assessed CT scans in the following manner. On the CT slice with the largest area of ICH, the largest diameter (A) of the haematoma was measured in centimetres. The dimension of the haemorrhage perpendicular to the largest diameter represented the second diameter (B) in centimetres. The height of the haematoma was calculated by multiplying the number of slices involved by the slice thickness, providing the third diameter (C). The three diameters were multiplied and then divided by two ($(A \times B \times C)/2$) to obtain the volume of ICH in cubic centimetres (Kothari et al. 1996). For the purpose of determination of diameter C, the first and last slices, where haematoma is first and last noted, were not counted. Additionally, IVH was defined as an intraventricular hyperdense image not attributable to calcification or the choroid plexus. MLS of the septum pellucidum was measured by a neuroradiologist blinded to the biomarker data and corrected for magnification using the scale provided on each CT image. MLS was calculated as the distance from the centre of the anterior horns of the lateral ventricles on the CT slice containing the third ventricle to a perpendicular line connecting the anterior and posterior insertions of the falx cerebri. A MLS of > 2 mm was considered significant.

Statistical methodology

Continuous variables are described by mean and standard deviation, and categorical variables with percentages. Association between biomarkers and radiographic outcomes (haematoma volume and MLS), analysed as continuous variables, were investigated with linear regression. Association between biomarkers and patient outcomes were investigated with linear regression analysis for the BI, which was analysed as a continuous variable, and logistic regression analysis for mRS, which was analysed as a dichotomous outcome (favourable vs unfavourable outcome). After investigating univariate associations, biomarkers were investigated together, and in combination with the ICH Score, to determine the increase in model predictive ability when additional variables are included. Multivariate model assumptions were tested. Additive effects were assessed with two-way interactions between covariates. Goodness of fit was assessed for logistic regression models with the Hosmer–Lemeshow test. Collinearity among predictors was assessed with tolerance and variance inflation tests. All analyses were performed with SAS version 9.3 or JMP 7.0.1.

Results

Baseline characteristics for subjects enrolled are shown in Table 1. BNP values were significantly correlated with the ICH Score ($r^2 = 0.42$, $p = 0.02$) and MLS ($r^2 = 0.42$, $p = 0.0002$) but not ICH volume ($r^2 = 0.14$, $p = 0.49$), LOS

Table 1. Characteristics of patients admitted after supratentorial intracerebral haemorrhage (ICH).

	Mean (SD)
Age (years)	62 ± 4.1
Gender (% male)	50.0
Race (% Caucasian)	57.12
ICH volume (cm^3)	30.65 (31.30)
Presence of IVH (%)	42.86
Latency to CT	9.32 (3.88)
MLS (mm)	5.04 (4.61)
GCS	10.46 (4.43)
NIHSS	15.19 (12.19)
ICH Score	1.57 (1.23)
mRS	3.39 (1.55)
BI	50.71 (37.11)
Hospital LOS	16.36 (28.40)
Peak BNP in 1st 24 h	236.41 (245.62)
Latency to highest serum BNP value	12.32 (15.81)
Peak S100B in 1st 24 h	203.63 (163.96)
Latency to highest serum S100B value	8.03 (12.7)

IVH, intraventricular haemorrhage; ICH volume, ICH lesional volume by computer tomography (CT); MLS, midline shift; GCS, Glasgow Coma Score; NIHSS, NIH Stroke Scale; mRS, modified Rankin Score; BI, Barthel Index; LOS, length of stay; BNP, brain natriuretic peptide; S100B, S100 calcium-binding protein B.

($r^2 = 0.02$) or initial GCS ($r^2 = 0.21$). S100B values were significantly correlated with ICH score ($r^2 = 0.39$, $p = 0.04$), MLS ($r^2 = 0.54$, $p < 0.0001$) and ICH volume ($r^2 = 0.48$, $p = 0.01$) but did not correlate with LOS ($r^2 = 0.01$) or initial GCS ($r^2 = 0.32$).

Univariate linear and logistic regression analysis demonstrated that BNP values were significantly associated with outcome, as measured by BI ($p < 0.0001$; slope 0.11, 95% confidence interval (CI) 0.69–0.15) and dichotomized mRS ($p = 0.04$; odds ratio 1.023, 95% CI 1.002–1.044); similarly, S100B values are predictive of outcome (BI, $p < 0.0001$, slope 0.16, 95% CI 0.09–0.22; mRS, $p = 0.02$, odds ratio 1.02, 95% CI 1.003–1.039).

To investigate the additional predictive ability that these biomarkers have beyond the ICH Score, we included them with the ICH Score in multivariable models predicting BI and dichotomized mRS. In a multivariable linear regression model predicting BI, ICH Score alone accounts for 44% of the variability in BI, as measured by the r^2 value. After including S100B and BNP in the model, the adjusted r^2 value increases to 0.66. Multivariable linear regression model for BI containing all three predictors is shown in Table 2. In a multivariable logistic regression model predicting favourable mRS outcome (Table 3), the ICH Score has a very high c-index, indicating good predictive ability (c-index = 0.86, 95% CI 0.69–1.01); after the addition of S100B and BNP, the c-index increases to 0.96 (95% CI 0.89–1.02). Although the comparison of the area under the ROC curves indicates that these are not statistically different in this small sample ($p = 0.13$), a trend is evident in the data. Furthermore, when the haemorrhage volume, the more traditional measure for functional outcome and an essential component of the ICH Score, is evaluated in this model, an r^2 value for the BI is determined to be 0.19, which is lower than for either biomarker alone or in combination, and a c-index for mRS is 0.87, which is similar to the ICH Score, BNP level and S100B level. Due to the high correlation between S100B and BNP ($r = 0.79$), a model constructed utilizing both biomarkers provides marginally increased predictive ability over using only one. These models are summarized in Table 4. However, when taken together the use of S100B and BNP levels suggests improved predictive value for neurological outcome at hospital discharge over traditional prognostic tools and when used in conjunction with these clinical scales improve the prognostic power than when either is used in isolation.

Table 2. Multivariable predictors of the Barthel Index.

	Slope	95% Confidence limits	*p*-Value
BNP	0.06	0.006–0.12	0.03
S100B	0.04	0.05–0.13	0.34
ICH Score	11.95	4.01–19.89	0.005

BNP, brain natriuretic peptide; S100B, S100 calcium-binding protein B; ICH, intracerebral haemorrhage. Adjusted R^2 value = 0.66.

Table 3. Multivariable predictors of favourable modified Rankin Score.

	Odds ratio	95% Confidence limits	*p*-Value
BNP	1.016	0.98–1.05	0.32
S100B	1.017	0.99–1.05	0.24
ICH Score	3.93	0.80–19.26	0.09

BNP, brain natriuretic peptide; S100B, S100 calcium-binding protein B; ICH Score, Intracerebral Haemorrhage Score. C-index = 0.96.

Discussion

Biological markers have been shown to correlate with outcomes in a number of different acute brain injury mechanisms, including traumatic brain injury (Biberthaler et al. 2002), ischaemic stroke (Reynolds et al. 2003) and subarachnoid haemorrhage (Stranjalis et al. 2007). Furthermore, it appears that after acute brain injury a panel of biomarkers may add potential benefit in terms of diagnostic precision and prognostic accuracy over any one biomarker alone (Laskowitz et al. 2005). Thus, a biomarker-based test may have utility in providing additional prognostic information when used in conjunction with traditional radiographic and clinical variables to guide early management decisions, as in global ischaemia after cardiac arrest (Ekmektzoglou et al. 2007, Sodeck et al. 2007) and as a surrogate endpoint in early clinical trials, such as in ischaemic infarction (Pettigrew et al. 2006) and subarachnoid haemorrhage (Lynch et al. 2005). To this end, our data demonstrate that both S100B and BNP are highly correlative with functional neurological outcome following primary

Table 4. Measures of model predictive ability for biomarkers for functional neurological outcome in patients after supratentorial intracerebral haemorrhage (ICH).

	Predictor							
Outcome	BNP	S100B	BNP + S100b	ICH score	ICH volume	BNP + S100b + ICH score	BNP + ICH score	S100B + ICH score
Barthel Index(adjusted r^2 value)[a]	0.54	0.50	0.58	0.44	0.19	0.66	0.66	0.61
Stratified mRS(c-index)[b]	0.87	0.89	0.91	0.86	0.87	0.96	0.96	0.94

BNP, brain natriuretic peptide; S100B, S100 calcium-binding protein B; ICH volume, ICH lesional volume by computer tomography; mRS, modified Rankin Score. [a]r^2 value represents the proportion of variability in the outcome measure accounted for by predictors in the model; [b]c-index value is a measure of the predictive ability of the model; 1.0 represents perfect.

supratentorial ICH at the time of hospital discharge and add additional predictive value over traditional prognostic scoring systems.

The calcium-binding protein, S100B, belongs to a family of microglial proteins found as dimers of two different subunits (alpha and beta) with types alpha–beta and beta–beta described as S100B protein. When acute structural damage occurs to microglia and Schwann cells, S100B is released into the cerebrospinal fluid and, with disruption of the blood–brain barrier, into the blood (Abraha et al. 1997). Therefore, not only is serum S100B reflective of glial injury but may also be indicative of blood–brain barrier dysfunction (Kanner et al. 2003). Furthermore, it is not affected by haemolysis and remains stable for several hours allowing for delayed but reliable serological analysis after acquisition. Finally, its short half-life makes measurements readily applicable to current pathophysiological state and vital to determination of intervention in acute care settings (Buttner et al. 1997).

BNP is a neurohormone produced as a pro-hormone (pro-BNP) comprising 108 amino acids (Valli et al. 1999). BNP is then enzymatically cleaved into physiologically active BNP and the amino-terminal portion of the pro-hormone after being released primarily from the cardiac ventricles in response to increased wall tension (Mukoyama et al. 1991). Serum BNP is typically elevated in patients with heart failure (Mukoyama et al. 1990), but BNP levels have been found to be rise early and rapidly after acute brain injury (Nakagawa et al. 2005). By means of its natriuretic and diuretic properties, this neurohormone produces a myriad of biological effects, such as vasodilatation (Laragh 1985), changes in electrolyte and fluid balances (Epstein et al. 1987) and inhibition of the sympathetic nervous system (Floras 1990). Although there is a paucity of data regarding BNP levels after ICH, recent reports demonstrate elevations after subarachnoid haemorrhage (Fukui et al. 2004, McGirt et al. 2004, Yarlagadda et al. 2006), traumatic brain injury (Sviri et al. 2006) and ischaemic stroke (Koenig et al. 2007, Nakagawa et al. 2005). Although preliminary data suggest that BNP elevations may be associated with an increase in cerebral blood flow after acute brain injury, it remains unclear whether this is an adaptive response related to increasing cerebral blood flow (Akdemir et al. 1997, Iida et al. 2001, Nogami et al. 2001) or a deleterious effect resulting in increased ischaemia (Sviri et al. 2000, 2003).

In patients suffering from ICH, few published data exist examining the prognostic role of serological markers. Relationships have been drawn between certain serum biomarkers in differentiating ischaemic from haemorrhagic stroke (Allard et al. 2004, Laskowitz et al. 2005) as well as prediction of early haematoma expansion, such as matrix metalloproteinases (Abilleira et al. 2003). However, the use of serological markers to determine functional outcome after ICH is currently limited to interleukin-11 (Fang et al. 2005) and S100B. Weglewski et al. (2005) demonstrated the time course over which S100B levels elevate and then decline to baseline following ICH. Furthermore, both early deterioration and long-term functional outcomes (3 months) were found to correlate with S100B levels on admission after ICH. However, Delgado et al. (2006) did not find that S100B provided a superior level of prediction when compared with initial ICH volume by CT, although utilization of mRS as their neurological endpoint may be less specific than BI. It is clear that the discovery of a serological marker(s) predictive of functional neurological outcome after ICH with high sensitivity and specificity is highly desirable.

Our data suggest that two biomarkers, S100B and BNP, are highly correlative with functional neurological outcome after ICH and that when either is used in conjunction with more traditional prognostic scoring systems, such as the ICH Score (Hemphill et al. 2001), there is an incremental increase in ability to predict prognosis. It appears that, mechanistically, these biomarkers may be related to enhanced inflammation, as demonstrated by their correlation with MLS, an accepted surrogate for cerebral oedema (James et al. 2009). Interestingly, the lack of correlation of S100B and BNP in our dataset with more traditional predictive variables, such as GCS and haematoma volume, may suggest that these serological proteins may be more reflective of a secondary inflammatory effect, rather than the initial injury due to mass effect. It is important to note, however, that our data do not demonstrate an improvement in predictive power by using both markers concordantly. In addition to determining the exact time course of elevation and underlying pathophysiological mechanisms represented by these markers, further studies should also address the possibility of utilizing biomarkers in differentiating the exact causes of ICH (primary, amyloid angiopathy, etc.).

There are several limitations of our data that should be addressed. First, this is a small pilot study and should not be used to dictate current clinical care strategies. We believe we have identified two useful and important biological markers that add to existing knowledge regarding prognosis after ICH; however, clinical utility needs to be borne out by further study. Next, outcome measures were assessed at discharge rather than in the post-rehabilitation period, allowing for the possibility of some patients initially dichotomized into unfavourable status to ultimately obtain higher functional condition after several months of rehabilitation. We believe this is less likely as both biomarkers correlated strongly with traditional prognostic tools, such as the ICH Score. Also, the use of the highest biomarker level within the first 24 h after ictus, rather than at a fixed time point, may have further biased the results, i.e. if less impaired patients with theoretically better prognoses missed the

peak biomarkers level due to discharge or, inversely, mortally injured patients died prior to peak levels. We feel that either circumstance is unlikely as there was a positive correlation with poor neurological outcome (including death) and protein level, and no patients died or were discharged within the first 24 h after entry to the study. Third, some of the patients who died in this study underwent withdrawal of care, which is fundamentally different from death in the setting of maximum therapeutic intervention. However, as ICH volume and GCS both correlate with each other and with outcome in our dataset, we believe it is likely that our results would be borne out in a long-term study to evaluate more definitive outcome measures. Finally, our values were followed only over the first 24 h after haemorrhage, and it is possible that there may be a second peak of serum values as inflammation increases during haemoglobin breakdown, which typically occurs several days after haemorrhage.

Although this study was not designed to examine the utility of repeated measurements, ultimately the utility of biological markers may finally rest in their ability to be followed over time, which is far easier than performing serial imaging studies. Despite the diagnostic and prognostic value of CT imaging for haemorrhage volume in the acute setting, there remains a significant degree of indecision regarding end of life decisions in the first 24–48 h after ICH. However, it appears that S100B and BNP elevation in the first 24 h after injury may augment existing technologies to assist in these difficult decisions.

In conclusion, S100B and BNP are highly correlated with functional neurological outcome at hospital discharge after supratentorial primary ICH. Furthermore, these biomarkers add prognostic information over traditional ICH scores that incorporate clinical and radiographic features. Further study on the exact time course of serological changes and their prognostic value for long-term functional and neurocognitive examination are indicated.

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