A 24 h Delay in the Redox Response Distinguishes the most Severe Stroke Patients from Less Severe Stroke Patients

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Abstract

Objective: Admission measures of stroke severity are often used to assess 30-90 day outcomes. Since some patients have a poor outcome by discharge, we sought to identify a biomarker that distinguishes severities as well as acute outcomes. Disruptions in the equilibrium of the redox system were used as an indicator of stroke severity and acute outcome.

Methods: Oxidation reduction potential (ORP), a measure of the redox system, was assessed in plasma at admission and 24 h later in patients admitted with stroke symptoms (n=76). Overall differences in ORP between stroke patients and healthy controls were determined. Within stroke patients, changes in ORP as a function of hospital discharge status, modified Rankin scale and NIHSS were assessed using ANOVA and the ability to predict acute outcome in ischemic stroke patients based on ORP was compared to NIHSS using Receiver Operator Characteristics.

Results: Stroke patients had higher ORP levels than healthy controls (p<0.05). Patients discharged to hospice or who died had significantly lower values at admission than those discharged to home or intermediate care facilities (p<0.05). However, in the following 24 h, these patients significantly increased their ORP measures (p<0.05). An ORP value >146.6 mV at admission was associated with a 46 fold increased odds of a good acute outcome.

Conclusion: ORP measured at admission identified patients which died or were discharged to hospice based on their lower ORP values. The lower ORP and subsequent increase 24 h later in the most severely affected patients may reflect a failure or a delay in engaging the redox system, a response that may, during acute stages, be beneficial.

Keywords: Ischemic stroke; Oxidative stress; Redox; NIHSS; Acute outcomes

Introduction

Approximately 795,000 people experience cerebral stroke per year in the USA [1]. Of those, 8-10% is expected to die in hospital [1,2]. Identifying patients most likely to have a fatal outcome during this acute period could assist health care providers in taking preventative actions.

Several stroke biomarkers have been used to predict 30-90 day outcomes based on serum levels of glial fibrillary acidic protein (GFAP), C-reactive protein (CRP), cortisol and glucose [3-5]. Only one study reported predictive values using in-hospital death as an end-point [6]. Serum glucose levels at admission reliably predicted 72 h mortality with a sensitivity of 88%; however admission glucose levels could not predict 7 day mortality [6]. Since the average hospital length of stay for a stroke patient is 6 days [1], it would be beneficial to have a biomarker that includes the hospitalization period.

Data suggest stroke patients are under a state of oxidative stress [7,8]. Increases in reactive oxygen species (ROS), lipid peroxidation, isoprostanes and decreases in antioxidant activity were associated with functional measures like the Modified Rankin Scale (MRS) or the NIH Stroke Score (NIHSS) [7,8]. We used a novel technology to assess oxidative stress by measuring oxidation-reduction potential (ORP) in patients with stroke symptoms. ORP, also known as the redox potential, is an inclusive index measuring the activity of all oxidants to all antioxidants, providing an assessment of the entire redox system [9]. Previous studies using similar technology revealed changes in ORP as a result of exercise, traumatic brain injury and multi-trauma [10-12]. We hypothesized that there are reliable changes in the redox system after stroke reflected by differences in ORP as a function of stroke

severity. Furthermore, these differences might be used for predicting acute outcome in stroke patients.

Methods

This study was conducted at Swedish Medical Center (SMC), a comprehensive stroke center located in Englewood, CO. Admitted patients who presented with stroke symptoms were consented into the study (N=162). Consent for participation was given by the patient or their legally authorized representative. The study was approved by the HealthOne Institutional Review Board and in accordance with institutional guidelines.

Patient inclusion criteria were consecutively admitted patients who presented with stroke symptoms between January 1, 2010 and December 31, 2011 and who were older or equal to 18 years of age. Patients were then excluded if they transferred into SMC (n=63) from another facility, did not have a blood sample available on the day of admission (n=4) or did not have a stroke or did not have a transient ischemic attack (TIA) (n=19). Final study group included 76 patients.

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Study procedures included up to once-daily collection of a blood sample during the patients' hospital stay, typically collected in the mornings. Venipuncture was carried out by a laboratory phlebotomist at the same time that blood was collected for diagnostic purposes. There were no other study procedures. Blood was processed to plasma, aliquoted and stored at -80°C.

Oxidation reduction potential (ORP)

ORP was measured using the RedoxSYS System (Aytu Bioscience, Englewood, CO), a galvanostatic-based technology [13]. The RedoxSYS analyzer, using a constant negligible current, measures the passive exchange of electrons between oxidants and reductants. This measure is the static ORP (sORP; millivolts, mV); a higher sORP suggests an imbalance in the redox system in favor of oxidant activity, also known as oxidative stress. The analyzer then applies an increasing oxidative current which measures the induced exchange of electrons. This measures the capacity ORP (cORP; microcoulombs, μ C) which is the ability of the biological sample to withstand an oxidative insult, also known as antioxidant capacity. In the current study, cORP data (μ C) were not normally distributed, therefore, the inverse cORP (*icORP*, μ C⁻¹) value was used, and thus a *higher icORP* suggests lower capacity to handle induced oxidative stress.

Both ORP measures were obtained from a single thawed room-temperature plasma sample (30 μ L). Each sample was measured in duplicate. ORP values were also measured in archival plasma samples from 10 self-declared, non-smokers, healthy volunteers.

Statistical analyses

sORP and *icORP* values on day of admission and their 24 h change were used to determine if changes in the redox system could distinguish between various severities of stroke based on discharge status, discharge MRS scores and admission NIHSS. Hospital discharge status was categorized as follows: Home (home or home health), Intermediate (skilled nursing or rehabilitation sites) and Hospice/Death. MRS scores were grouped based on actual values (0-6). NIHSS measures were assembled into four groups: scores 0-4: minor, 5-15: moderate, 16-20: moderately severe and 21-42: severe.

Additional variables of interest for ischemic stroke (IS) patients were etiology, tissue plasminogen activator (tPA) treatment and reperfusion therapy- intravenous and/or arterial thrombolysis (IV and/or IA). Age, sex, time between symptom onset and arrival and infectious complications were also analyzed in all stroke patients for differences in sORP and *icORP* at admission and 24 h change.

Data were analyzed using Student's t-test for two groups, ANOVA for more than two groups, Pearson's correlation, or Pearson's Chi square for proportions. Appropriate *post-hoc* analyses were done when a significant effect was found ($p \leq 0.05$). A Receiver Operator Characteristic (ROC) statistic was used to identify admission sORP and NIHSS measures that best forecasted acute outcome dichotomized into a good acute outcome (patients discharged to home or intermediate care facilities) and poor acute outcome (patients transferred to hospice or who died in-hospital). The optimal cut-off criterion for predicting discharge status was identified using the Youden index for both variables. Data was analyzed using Statistica 13 (Dell, Inc.) and MedCalc Statistical Software 15.8. All statistics are presented within their respective graphs or within the text if not graphed. Data represent the mean $\pm$ SEM or as otherwise noted in the tables.

Results

The final investigative group included 76 stroke patients; 49 with

IS, 9 with hemorrhagic stroke, and 18 with TIA. The average length of hospital stay for the home discharge group was 4 days, intermediate care group was 9.3 days and hospice/death group was 3.8 days. The prevalence of mortality (in-hospital death and those transferred to hospice) in the study group was 7.9%, similar to previous reports [2]. General patient demographics are presented in Table 1.

Intra-patient reliability in ORP measures obtained from blood plasma samples was assessed by comparing replicate means and by correlation. There were no significant differences in sORP ($t(150)=1.10$, $p>0.05$) or in *icORP* ($t(148)=0.51$, $p>0.05$) between replicates. The correlations between replicates were significant with $r=0.98$ ($R^2=0.96$, $p<0.05$) and 0.88 ($R^2=0.77$, $p<0.05$) for sORP and *icORP* measures respectively. Because of the high level of reliability, further ORP analyses were performed on the mean value of the replicates.

All stroke patients vs. control volunteers

Archival control samples were significantly younger than the overall stroke population ($t(84)=4.64$, $p<0.001$), thus stroke data were grouped into an age-matched stroke cohort and an unmatched older stroke cohort. Analyses of sORP and *icORP* values, using admission values for stroke cohorts, indicated that both age-matched and unmatched stroke patients had higher sORP and *icORP* values than controls ($p<0.05$; Figure 1) demonstrating that both stroke groups had elevated oxidative stress measures compared to normal controls.

There were no differences in sORP or *icORP* values between the

	N	Mean $\pm$ sem	Median (min-max)
Gender (% male)	76 (38.2%)		
Ethnicity (% Caucasian)	76 (85.5%)		
Age (years)	76	73.0 $\pm$ 1.83	78 (26-94)
Stroke Type	76		
Hemorrhagic (hypertensive, cerebral amyloid angiopathy, aneurysm)	9		
Transient Ischemic Attack (TIA)	18		
Ischemic	49		
Cardioembolism	28		
Large vessel atherosclerosis (LVA)	8		
Small vessel atherosclerosis (SVA)	5		
Other (vessel dissection, cryptogenic, hypercoagulability)	8		
Time from Symptom Onset to Arrival	68	4.36 $\pm$ 0.92	1.41 (0.33-42.9)
<2 h	48		
2-4 h	19		
4-6 h	5		
6-12 h	4		
>12 h	9		
tPA treatment	49		
None	32		
Yes (time to treat in minutes)	17	91.7 $\pm$ 5.42	88 (61-135)
Hospital length of stay (days)	74	6.1 $\pm$ 0.84	4 (1-44)
NIHSS at admission	64	7.3 $\pm$ 0.88	5 (0-25)
Infectious Complication	95		
No	84		
Yes (pneumonia, MRSE and/or UTI)	11		

Table 1: Patient demographics and sample sizes (N).

type of stroke or between the IS etiologies ($p > 0.05$; Figures 2 and 3 and Table 1), thus at admission and after 24 h, all stroke patients had similar levels of oxidative stress despite the type or cause of the stroke.

Discharge status and mortality in all stroke patients

Admission sORP values were higher in patients discharged to home or intermediate care facilities compared to those who died in-hospital or transferred to hospice ($p < 0.05$; Figure 4a), signifying a lower state of oxidative stress in this group with a poor acute outcome. In 24 h, however, the death/hospice patients had a significant increase in sORP which was not observed in the other groups ($p < 0.05$; Figure 2b). This change brought the poor acute outcome group's sORP values to the level measured in the other less severe group's 24 h later ($p > 0.05$, data not shown).

Admission icORP values were also higher in patients discharged to home or intermediate care ($p < 0.05$; Figure 4c) compared to those who would be discharged to hospice or died. Higher icORP values are indicative of lower antioxidant capacity. In 24 h, the hospice/death group again had a significant increase in their icORP values ($p < 0.05$; Figure 2d), such that by this time there were again no significant differences between the groups ($p > 0.05$, data not shown).

Because those with a poor acute outcome were significantly lower, *ad-hoc* analyses between the archived healthy controls and those who died or went to hospice were done and found no significant differences in admission sORP ($t(14) = 0.62$, $p = 0.54$) but a significant increase icORP ($t(14) = 3.58$, $p < 0.001$). This comparison would suggest that the fatal acute outcome patients were more similar to the control volunteers in sORP values than the other stroke patients at admission and their icORP tended to be in between the two groups.

Of the patients who died or went to hospice, the poor acute outcome group, 100% (6/6 patients; specificity) had an admission sORP ≤ 142.9 mV. Of those that went home or were discharged to an intermediate care facility, 78.6% (55/70 patients; sensitivity) had a sORP > 142.9 mV. The odds ratio that sORP values at admission ≤ 142.9 mV would result in a good outcome was 46.5 ($p < 0.005$). ROC analysis for this cut-off covered a significant area under the curve (AUC) of 0.92

for all stroke patients and 0.89 for the IS and TIA subgroup ($p < 0.05$). Based on these data and similar reported prevalence rates [1,2], we calculated the predictive values for estimating a non-fatal discharge status (home or intermediate care) for all patients and for IS/TIA patients with the result that both were 100% (Table 2). Since the length of stay in the hospital after admission for each group ranged from 1-44 days for home/intermediate care and 1-8 days for hospice/death, sORP measured at admission predicted a non-fatal acute outcome 24 h and up to 44 days, in advance.

Stroke severity

All stroke patients with a discharge MRS of 5-6 had lower sORP and icORP values at admission than those with scores 0-4 ($p < 0.05$; Figure 5). Thus, patients with the most severe strokes had low levels of oxidative stress (sORP) and better antioxidant capacities (icORP) initially. In 24 h, patients with MRS of 5-6 had a large elevation in both sORP and icORP (Figure 5), which brought the values up to similar levels as those in the MRS groups 0-4 within the following 24 h ($p > 0.05$, data not shown).

In the IS and TIA subgroup, the most severe stroke group (NIHSS 21-42) also had lower admission sORP and icORP values compared to the minor, moderate, or moderately severe groups ($p < 0.05$; Figure 6). The moderately severe group had the highest sORP values at admission ($p < 0.05$). During the next 24 h, changes in sORP and icORP were not different; however enough change occurred in this time to permit the severe group to reach values that were comparable to the other groups.

To compare the predictive differences between sORP and NIHSS on acute outcome, a ROC analysis for NIHSS was also performed. The AUC for NIHSS taken at admission was 0.86 ($p < 0.05$) and was not significantly different from the AUC found for sORP ($p > 0.05$). A cut-off NIHSS value of ≤ 6 was identified as the optimal criterion. A 100% of those with a poor acute outcome (3/3; specificity) were above this cut-off but only 63.9% of those with a good acute outcome were below it (39/61; sensitivity). Overall, the positive and negative predictive values were similar but NIHSS was slightly less accurate than admission sORP values (Table 2).

Figure 1: Stroke patients (all types) had higher sORP and icORP values than controls. Stroke patients age-matched to controls had higher sORP (A) and icORP values (B) Unmatched stroke patients, which were older, were also higher than controls values. The younger matched stroke patients had significantly higher sORP values than the older unmatched stroke patients, but they were not different from each other in icORP. Sample sizes were Controls (n=10), Matched Stroke (n=18) and Unmatched Stroke (n=61) *significantly greater than Controls; **significantly greater than Controls and Unmatched Stroke; $p < 0.05$.

Figure 2: Different types of stroke had similar levels of oxidative stress. **A:** sORP values at admission were similar between ischemic, hemorrhagic, and TIA patients. **B:** In 24 h, the changes in sORP were not significantly different although there was a trend for the hemorrhagic patients have elevated sORP values more so than the other groups. **C/D:** icORP did not differentiate between stroke types at admission or in the subsequent 24 h.

Figure 3: Differences in the redox measures did not discriminate between different IS etiologies. **A/B:** IS patients with a cardioembolism (Card), large vessel atherosclerosis (LVA), small vessel atherosclerosis (SVA), or other source (i.e., vessel dissection, hypercoagulation etc.) had similar measures of sORP at admission and which did not change much in the succeeding 24 h. **C/D:** icORP values also changed very little between groups at admission and in the next 24 h.

Figure 4: An acute outcome of hospice/death was related to diminish oxidative stress at admission for all stroke patients. **A:** Patients discharged to hospice or who died had lower sORP values at admission than patients with a better outcome, discharged to home or an intermediate care facility. **B:** A significant increase in sORP values occurred in the next 24 h in that same poor outcome group. **C:** icORP values at admission were also lower for the patients experiencing hospice/death at discharge compared to the other groups, indicating a higher level of antioxidant capacity at admission. **D:** Changes in icORP indicate that those discharged to hospice or who died in-hospital had an increase not measured in the other stroke patients, suggesting a loss of capacity in the next 24 h. Relevant statistics are presented within each graph. Sample sizes were Home (n=40), Intermediate (n=30), Hospice/Death (n=6) *significantly different from all other groups, p<0.05.

	Admission sORP		Admission NIHSS
	All Stroke Patients (N=76)	IS and TIA Patients (N=67)	IS and TIA Patients (N=64)
AUC (95% CI)	0.92 * (0.83-0.97)	0.89 * (0.79-0.95)	0.86 * (0.75-0.93)
Sensitivity (95% CI)	78.6 (67.1-87.5)	78.1 (66.0-87.5)	63.9 (50.6-75.8)
Specificity (95% CI)	100 (54.1-100)	100 (29.2-100)	100 (29.2-100)
Positive Predictive Values (95% CI)	100 (93.5-100)	100 (92.9-100)	100 (91.0-100)
Negative Predictive Value (95% CI)	28.6 (11.3-52.2)	17.6 (3.8-43.4)	12.0 (2.5-31.2)
Accuracy	80%	79%	66%

Table 2: Comparison of ROC analyses between sORP cutoff at admission (>142.9 mV) and the NIHSS cut off (≤ 6) for identifying and predicting a positive outcome at time of hospital discharge (home or intermediate care).

TPA Treatment, reperfusion and remaining variables

TPA treatment was given to 17 IS and two TIA patients, with a mean time to treat of 92 min (min-max: 61-135 min). sORP at admission and its 24 h difference did not change as a function of tPA treatment (Figure 7). Admission icORP values also did not distinguish between those given tPA and those that were not. However, in those that did get tPA treatment, a significantly negative change in icORP during the subsequent 24 h was measured (p<0.05), suggesting an acquisition of antioxidant capacity in 24 h compared to a loss or no change in antioxidant capacity in the untreated stroke group. The distribution of tPA use was proportionate and met expected frequencies across IS etiologies, NIHSS groups, MRS and discharge statuses ($\chi^2=1.96, 7.56, 4.15, 0.05$ respectively, p>0.05 for all) suggesting no interactive effects with the above results.

Nineteen patients also received some type of reperfusion therapy, either intravenous thrombolysis (IV; n=14), intra-arterial thrombolysis (IA; n=2) or both (IA/IV; n=3). At admission, there no differences in sORP or icORP values between treatment groups (Figure 8). After treatment, during the subsequent 24 h, there were significant changes with patients that received any kind of IA therapy with an increase in sORP value, whereas those with no therapy or IV only had minimal or no change in sORP. In contrast, those given IV therapy had a significant drop in icORP values in the subsequent 24 h, signifying a gain in antioxidant capacity (Figure 8). The distribution of patients receiving reperfusion therapy was proportionate across discharge, MRS, ischemic etiology, and NIHSS groups ($\chi^2=2.13, 9.03, 6.03$ and 12.26, respectively, p>0.05 all), suggesting that the small changes induced by reperfusion therapy did not alter acute outcomes.

Differences in sORP and icORP were not significant as a function

Figure 5: The highest MRS scores had the lowest measures of oxidative stress at admission. **A:** All stroke patients with a discharge MRS score of 5 or 6 had lower sORP values at admission than those with MRS scores between 0-4. **B:** In 24 h, those with the highest MRS score (5-6) had a significant elevation in sORP measures. **C:** Antioxidant capacity was higher in those with an MRS score of 5 or 6, indicated by lower icORP values compared to those with an MRS score of 0-4. **D:** As with sORP, 24 h resulted in a significant loss in antioxidant capacity in the most severe patients, as revealed by increased icORP values for those with MRS score of 5 or 6. Sample size based on MRS scores 0 (n=20), 1 (n=13), 2 (n=11), 3 (n=15), 4 (n=19), 5 (n=3), 6 (n=4). Relevant statistics are presented within each graph. ^, significantly different from Scores 0 and 2; * significantly different from all other scores; p<0.05.

of sex, time between symptom onset and arrival, or infectious complications ($p>0.05$ for all; data not shown). These variables were also proportionately distributed across discharge status ($\chi^2=0.67$, 4.83 and 1.25 respectively, $p>0.05$ for all).

Discussion

This study supports two major conclusions. First, the most severely affected stroke patients (death/hospice, MRS 5-6 and/or NIHSS ≥ 21) have lower measures of oxidative stress at admission than less severe stroke patients, suggesting these patients are not under the state of oxidative stress that would be expected. However, the 24 h change in sORP and icORP suggests a delayed redox system response to stroke in the most severely affected stroke patients, as both ORP values increased during this time. Secondly, that difference in admission sORP values could project future discharge status (acute outcomes) of stroke patients.

Contrary to traditional views, a redox imbalance in favor of oxidative stress can be beneficial [14-16] and failure or a delay in engaging the redox system to oxidative stress may be an important indicator of outcome [17]. In terms of stroke, oxidative stress has important roles in vascular remodeling and inflammatory responses.

Cerebral vascular tone is adjusted through the production of oxygen-based radicals via the activity of NADPH-oxidase enzymes (NOX) [18-20]. In rat models of stroke, NOX, and thus oxygen-based radicals, increase within hours of occlusion and co-localize with angiogenesis [20,21]. A delayed response in the redox system, as measured in our most severely affected stroke patients, could result in postponed vascular and redox compensations thus increasing ischemic brain damage and consequentially in poor acute outcomes [22].

The generation of oxidative stress and its modulation of the inflammatory response is commonly viewed as a deleterious cycle. Most studies report that elevations in the immune response are associated with higher NIHSS values and poorer outcomes however these studies omitted the most severe stroke patients by looking at long-term outcomes and thus removing those patients who died in hospital or by grouping NIHSS moderately severe and severe groups together (NIHSS ≥ 14) [4,23,24]. It is possible that they missed observing changes in the immune system on the most severe stroke patients with the poor acute outcomes. Several studies without these limitations have shown that those patients with the highest NIHSS at admission and largest infarct volumes initially had significantly lower monocyte HLA-DR expression, fewer T-cells, fewer T-helper cells, fewer cytotoxic T-cells and lower interferon gamma (INF- γ) expression and elevated cortisol

Figure 6: In IS patients, these were NIHSS group (≥ 21) had lower measures of oxidative stress than the less severe NIHSS groups. **A:** IS and TIA patients with NIHSS scores falling into the severe level (NIHSS ≥ 21) had significantly lower sORP values, whereas those with a moderately severe (mod/severe; NIHSS 16-20) level had slightly higher sORP values. **B:** While there was some change in sORP in 24 h post-admission, this did not reach significance. **C:** The severe stroke group also had smaller admission icORP values than the other groups. **D:** Changes in icORP during the 24 h period post-admission did not reach significance. Sample sizes based on NIHSS groups were Minor (NIHSS 0-4; n=28), Moderate (NIHSS 5-15; n=24), Mod/Severe (NIHSS 16-20; n=8) and Severe (NIHSS ≥ 21 ; n=4). Relevant statistics are presented within each graph. [^]significantly greater than Severe and Moderate groups; *significantly different from all other groups, p<0.05.

[5,25,26]. In the current data set, an attenuation of the oxidative stress response was recorded and because ROS modulates the immune response, it is possible that a low sORP at admission could be an early indicator for immunodepression in stroke [27,28].

As an indicator of the redox system, the initial sORP levels provided insight into the outcome of stroke patients, with those having higher sORP values at admission, greater than 142.4 mV, most likely to have a more favorable acute outcome (discharge to home or intermediated facility) compared to those with a low sORP (less than 142.4) having a poor acute outcome (discharge to hospice or death). In our study, we compared the predictive ability of sORP admission levels to admission NIHSS and found that sORP had better predictive statistics, including a better accuracy (Table 2). Earlier work evaluating the predictive power of NIHSS on outcome has produced similar values found to the current study, with AUCs between 0.78 and 0.90, 88-91% of good outcomes correctly identified and 69-88% of poor outcomes identified [29-32]. The variability in the NIHSS predictive power between studies most likely reflects different NIHSS cut-off values and differentially defined outcomes, although it is possible that the variability lies in the administration of the NIHSS. Administration of the NIHSS requires a certified rater, yet despite this, there can be large variation in scoring with ataxia and facial weakness having the highest rate of subjective interpretation [33,34]. Measuring sORP is objective and requires no

special certification, thus the fact that the sORP is at least as reliable as NIHSS and at most more reliable than NIHSS, measuring sORP is an unbiased and accessible indicator of acute outcome in stroke.

Biomarkers, GFAP, CRP, cortisol and glucose, are all unbiased measures that have been used to predict post-hospital (30-90 days) mortality [3-5] rather than more acute, in-hospital outcomes. Since 8-10% of stroke patients will die in-hospital [1,2] and 84% of hospice patients die with a median length of stay of 13 days [35] we felt that predicting early mortality was an important event. It is unknown if measuring GFAP or CRP could predict a more immediate outcome, however, these markers are not adapted to the rapid turnaround times needed to estimate in-hospital mortality. The potential prognostic value provided by sORP measures could be used in the decision making processes regarding treatment with the intent to prevent predicted mortality.

Unfortunately, how to proceed and with what treatment is still debated. TPA, an accepted FDA approved drug for IS, has a 3 h window of effectiveness from the time of symptom onset [36,37]. In the current study, 30% of IS patients were outside this window, only 36% received tPA and tPA treatment was not associated with a better acute outcome. There was an increase in antioxidant capacity after tPA treatment in the 24 h period after admission, however this change was not associated with acute outcomes. Previously, in less severe stroke patients, lower

Figure 7: Antioxidant capacity increases in those given tPA treatment the 24 h period after admission. **A/B/C.** sORP and icORP values were similar between those IS and TIA patients that received tPA treatment and there was no difference in how sORP changed in the following 24 hours. **D.** In those given tPA treatment, there was a significant decrease in icORP values, indicating an increase in antioxidant capacity during next 24 h, which was not seen in those that were not given tPA treatment. *significantly less than the No tPA treatment group, $p < 0.05$.

Figure 8: Reperfusion therapy changes the redox balance. **A/C.** There were no significant differences in admission ORP values between IS and TIA patients given IV, IA or IA and IV (IA/IV) reperfusion therapy from those that did not. **B.** 24 h after admission, those given IA or IA/IV therapy had a significant increase in sORP compared to IV or untreated patients. **D.** In contrast, those given IV therapy had a significant decrease in icORP values during the following 24 h, suggesting a gain in antioxidant activity during this time. *significantly different from the remaining groups, $p < 0.05$.

oxidative stress and tPA treatment were associated with better long-term outcomes [7,8,37]. Similar to tPA treatment, IV reperfusion therapy in the current study was associated with increased antioxidant capacity in the period post-treatment, but it was not associated with a better acute outcome despite previous studies indicating its clear benefit in long-term outcomes [38].

Several studies report beneficial effects of antioxidant-based treatments given outside the tPA window, such as edaravone within 72 h, ebselen within 24 h or lipophilic statins anytime between 1-10 days [39-42]. These treatments support the notion that reducing oxidative stress in stroke could be beneficial [39,40,43]. Our study confirms that in general, stroke patients are under a state of oxidative stress compared to healthy controls. Further, we endorse the idea that oxidative stress increases somewhat with stroke severity [7,8]; however, the most severe stroke patients, those discharged to hospice/death, with MRS of 5-6 or NIHSS scores >21 had lower sORP measures, compared with other less severe stroke patients. The fact that this has not been reported before might be due to previous studies investigating longer outcomes (30 or 90 days) [7,8,39-42], thereby eliminating the most severely affected stroke patients- those who die in-hospital or shortly thereafter. Therefore, studies suggesting that antioxidant treatment may benefit stroke patients are limited to those with a non-fatal acute outcomes and it remains unknown if it would benefit or harm patients with the most severe stroke and delayed redox response.

Conclusion

Data suggests that the redox system may need to favor oxidative stress at the outset of stroke to have a better acute outcome at hospital discharge. The failure to generate a timely oxidative stress response could result in a poor or fatal outcome by hospital discharge, as our data suggests that low sORP at admission is associated with poor acute outcomes. Once outside the acute outcome window, high oxidative stress does seem to be associated with poor post-hospital outcomes (≥ 30 days). Finally, treatment decisions, especially if considering an antioxidant approach, ought to be made with the understanding of the oxidative state of the patient prior to administration, as these therapies are currently untested in the most severe stroke patients with a low or delayed oxidative stress response.

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References

- Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, et al. (2015) Heart disease and stroke statistics--2015 update: A report from the American Heart Association. *Circulation* 131: e29-322.
- Ovbiagele B (2010) Nationwide trends in in-hospital mortality among patients with stroke. *Stroke* 41: 1748-1754.
- Xiong L, Yang Y, Zhang M, Xu W (2015) The use of serum glial fibrillary acidic protein test as a promising tool for intracerebral hemorrhage diagnosis in Chinese patients and prediction of the short-term functional outcomes. *Neurol Sci* 36: 2081-2087.
- Rocco A, Ringleb PA, Grittner U (2015) Follow-up C-reactive protein level is more strongly associated with outcome in stroke patients than admission levels. *Neurol Sci* 36: 2235-2241.
- Zi WJ and Shuai J (2013) Cortisol as a prognostic marker of short-term outcome in Chinese patients with acute ischemic stroke. *PLoS One* 8: e72758.
- Nardi K, Milia P, Eusebi P, Paciaroni M, Caso V, et al. (2012) Predictive value of admission blood glucose level on short-term mortality in acute cerebral ischemia. *J Diabetes Complications* 26: 70-76.
- Nanetti L, Raffaelli F, Vignini A, Perozzi C, Silvestrini M, et al. (2011) Oxidative stress in ischaemic stroke. *Eur J Clin Invest* 41: 1318-1322.
- Seet RC, Lee CY, Chan BP, Sharma VK, Teoh HL, et al. (2011) Oxidative damage in ischemic stroke revealed using multiple biomarkers. *Stroke* 42: 2326-2329.
- Costantini D, Verhulst S (2009) Does high antioxidant capacity indicate low oxidative stress? *Functional Ecology* 23: 506-509.
- Rael LT, Bar-Or R, Salottolo K, Mains CW, Slone DS, et al. (2009) Injury severity and serum amyloid A correlate with plasma oxidation-reduction potential in multi-trauma patients: A retrospective analysis. *Scand J Trauma Resusc Emerg Med* 17: 57.
- Rael LT, Bar-Or R, Mains CW, Slone DS, Levy AS, et al. (2009) Plasma oxidation-reduction potential and protein oxidation in traumatic brain injury. *J Neurotrauma* 26: 1203-1211.
- Stagos D, Goutzourelas N, Bar-Or D, Ntontou AM, Bella E, et al. (2015) Application of a new oxidation-reduction potential assessment method in strenuous exercise-induced oxidative stress. *Redox Rep* 20: 154-162.
- Rael LT, Bar-Or R, Kelly MT, Carrick MM, Bar-Or D (2015) Assessment of oxidative stress in patients with an isolated traumatic brain injury using disposable electrochemical test strips. *Electroanalysis* 27: 2567-2573.
- Scudellari M (2015) The science myths that will not die. *Nature* 528: 322-325.
- Watson JD (2014) Type 2 diabetes as a redox disease. *Lancet* 383: 841-843.
- Teodoro JS, Rolo AP, Palmeira CM (2013) The NAD ratio redox paradox: Why does too much reductive power cause oxidative stress? *Toxicol Mech Methods* 23: 297-302.
- Zhi L, Hu X, and Han C (2014) Biphasic changes (over reduction and over oxidation) of plasma redox status and clinical implications in early stage of severe burns. *J Crit Care* 29: 1063-1068.
- Karimi Galougahi K, Ashley EA, Ali ZA (2016) Redox regulation of vascular remodeling. *Cell Mol Life Sci* 73: 349-363.
- Miller AA, Drummond GR, Schmidt HH, Sobey CG (2005) NADPH oxidase activity and function are profoundly greater in cerebral versus systemic arteries. *Circ Res* 97: 1055-1062.
- Taylor CJ, Weston RM, Dusting GJ, and Roulston CL (2013) NADPH oxidase and angiogenesis following endothelin-1 induced stroke in rats: Role for nox2 in brain repair. *Brain Sci* 3: 294-317.
- Sobey CG, Heistad DD, Faraci FM (1997) Mechanisms of bradykinin-induced cerebral vasodilatation in rats. Evidence that reactive oxygen species activate K⁺ channels. *Stroke* 28: 2290-2294.
- Terpolilli NA, Kim SW, Thal SC, Kataoka H, Zeisig V, et al. (2012) Inhalation of nitric oxide prevents ischemic brain damage in experimental stroke by selective dilatation of collateral arterioles. *Circ Res* 110: 727-738.
- Emsley HC, Smith CJ, Gavin CM, Georgiou RF, Vail A, et al. (2003) An early and sustained peripheral inflammatory response in acute ischaemic stroke: Relationships with infection and atherosclerosis. *J Neuroimmunol* 139: 93-101.
- Idicula TT, Brogger J, Naess H, Waje-Andreassen U, Thomassen L (2009) Admission C-reactive protein after acute ischemic stroke is associated with stroke severity and mortality: The 'Bergen stroke study'. *BMC Neurol* 9: 18.
- Urrea X, Cervera A, Villamor N, Planas AM, Chamorro A (2009) Harms and benefits of lymphocyte subpopulations in patients with acute stroke. *Neuroscience* 158: 1174-1183.
- Haeusler KG, Schmidt WU, Foehring F, Meisel C, Guenther C, et al. (2012) Immune responses after acute ischemic stroke or myocardial infarction. *Int J Cardiol* 155: 372-377.
- Wink DA, Hines HB, Cheng RY, Switzer CH, Flores-Santana W, et al. (2011) Nitric oxide and redox mechanisms in the immune response. *J Leukoc Biol* 89: 873-891.
- Nayernia Z, Jaquet V, Krause KH (2014) New insights on NOX enzymes in the central nervous system. *Antioxid Redox Signal* 20: 2815-2837.
- Jeng JS, Huang SJ, Tang SC, Yip PK (2008) Predictors of survival and

- functional outcome in acute stroke patients admitted to the stroke intensive care unit. *J Neurol Sci* 270: 60-66.
30. Mansour OY, Megahed MM, and Abd Elghany EHS (2015) Acute ischemic stroke prognostication, comparison between Glasgow Coma Score, NIHSS Scale and Full Outline of UnResponsiveness Score in intensive care unit. *Alexandria Journal of Medicine* 51: 247-253.
 31. Sablot D, Belahsen F, Vuillier F, Cassarini JF, Decavel P, et al. (2011) Predicting acute ischaemic stroke outcome using clinical and temporal thresholds. *ISRN Neurol* 2011: 354642.
 32. Schaefer PW, Pulli B, Copen WA, Hirsch JA, Leslie-Mazwi T, et al. (2015) Combining MRI with NIHSS thresholds to predict outcome in acute ischemic stroke: Value for patient selection. *AJNR Am J Neuroradiol* 36: 259-264.
 33. Hills NK, Josephson SA, Lyden PD, Johnston SC (2009) Is the NIHSS certification process too lenient? *Cerebrovasc Dis* 27: 426-432.
 34. Lyden P, Raman R, Liu L, Grotta J, Broderick J, et al. (2005) NIHSS training and certification using a new digital video disk is reliable. *Stroke* 36: 2446-2449.
 35. Caffrey C, Sengupta M, Moss A, Harris-Kojetin L, Valverde R (2011) Home health care and discharged hospice care patients: United States, 2000 and 2007. *Natl Health Stat Report*: 1-27.
 36. Hebert M, Lesept F, Vivien D, and Macrez R (2016) The story of an exceptional serine protease, tissue-type plasminogen activator (tPA). *Rev Neurol (Paris)* 172: 186-97.
 37. Zivin JA (2009) Acute stroke therapy with tissue plasminogen activator (tPA) since it was approved by the U.S. Food and Drug Administration (FDA). *Ann Neurol* 66: 6-10.
 38. Hlavica M, Diepers M, Garcia-Esperon C, Ineichen BV, Nedeltchev K, et al. (2015) Pharmacological recanalization therapy in acute ischemic stroke - evolution, current state and perspectives of intravenous and intra-arterial thrombolysis. *J Neuroradiol* 42: 30-46.
 39. Otomo E (2003) Effect of a novel free radical scavenger, edaravone (MCI-186), on acute brain infarction. Randomized, placebo-controlled, double-blind study at multicenters. *Cerebrovasc Dis* 15: 222-229.
 40. Flint AC, Kamel H, Navi BB, Rao VA, Faigles BS, et al. (2012) Statin use during ischemic stroke hospitalization is strongly associated with improved post-stroke survival. *Stroke* 43: 147-154.
 41. Song B, Wang Y, Zhao X, Liu L, Wang C, et al. (2014) Association between statin use and short-term outcome based on severity of ischemic stroke: A cohort study. *PLoS One* 9: e84389.
 42. Yamaguchi T, Sano K, Takakura K, Saito I, Shinohara Y, et al. (1998) Ebselen in acute ischemic stroke: A placebo-controlled, double-blind clinical trial. Ebselen Study Group. *Stroke* 29: 12-17.
 43. Amaro S, Llull L, Renú A, Laredo C, Perez B, et al. (2015) Uric acid improves glucose-driven oxidative stress in human ischemic stroke. *Ann Neurol* 77: 775-783.