RESEARCH ARTICLE

The Stress-Immune Response in Acute Ischemic Stroke in Hyperglycemic Patients

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Abstract

Background and Hypothesis: Diabetic and hyperglycemic stroke patients have worse clinical outcome, higher mortality rates, larger infarcts, and more severe neurological disability than euglycemic individuals. The reasons for the worse outcome in diabetes are poorly understood, but may not be fully explained by hyperglycemia alone. Therefore, the objective of this prospective clinical study was to explore whether there is an association between the stress-immune response and clinical outcomes in the hyperglycemic state.

Methods: Circulating immune cells including activated neutrophils, monocytes, B-cells, NK cells and subpopulations of T-lymphocytes were measured by flow cytometry over 96 hours post stroke in both hyperglycemic and euglycemic patients. Cortisol, C-reactive protein (hs-CRP), HbA1C, and blood glucose were measured in central laboratory daily over 4 days in the acute post-stroke phase. Short- and long-term neurological outcomes were assessed by the National Institute of Health Stroke Scale (NIH-SS), modified Ranking scale (mRS) and discharge disposition. Discharge dispositions were captured at discharge and 90 days post stroke. Data on infection rates and all course mortality were collected during the hospital stay and at 3 months follow up.

Results: Hyperglycemic stroke patients had worse clinical outcomes, more in-hospital infections, less favorable discharge dispositions and higher mortality rates. Hyperglycemic individuals had higher cortisol and hs-CRP levels, higher peripheral neutrophil and monocytes concentrations and suppressed T- lymphocyte, B-cells and NK cells levels compared to their euglycemic counterparts.
Conclusion: Our data suggests an altered stress-immune response in the hyperglycemic state following acute ischemic stroke which could contribute to the poor clinical outcome in this high risk group.

Key words: Diabetes, hyperglycemia, ischemic stroke, lymphocytes, neutrophils

Introduction

Diabetic patients are a high-risk group after acute ischemic stroke, having a three-fold increased probability of recurrent stroke, a greatly enhanced stroke morbidity, and a three-fold increased stroke mortality compared to euglycemic patients (1). The reasons for the worse outcome in diabetic stroke patients remain understudied. In a recent clinical trial, aggressive control of hyperglycemia did not improve clinical outcome in stroke patients (2), suggesting that other mechanisms undely the poor clinical outcome of this high risk population.

Previous studies in our laboratory and by others have shown that endothelial activation, mass infiltration of leukocytes into the brain and an accumulation of inflammatory mediators occur within a few hours after an ischemic stroke, and can exacerbate the ischemic brain injury (3, 4). Leukocytes are considered the first line of defense to injury and have been implicated in mediating the immune response to ischemic stroke (5). In clinical stroke studies it has been observed that increased stroke severity and larger infarct volumes are associated with elevated neutrophil counts, as well as total leukocyte counts in peripheral blood samples, but not with lymphocyte levels (6). Furthermore, an increased neutrophil to lymphocyte(NL-ratio) ratio in the peripheral circulation has been shown to be an indicator of systemic inflammation and has been correlated with unfavorable outcomes in patients with acute myocardial infarction and acute ischemic stroke (7, 8)

In addition, the production of inflammatory chemokines and cytokines, such as TNF-α, IL-17, IL-1β and cell adhesion molecules such as P-selectin and ICAM-1, and small molecules such as prostanoids and leukotrienes are substantially elevated after stroke (9, 10).

Stress and the upregulation of pro-inflammatory cytokines following stroke stimulate the hypothalamic-pituitary-adrenal (HPA) axis and enhance sympathetic nervous system activity. Increased serum cortisol levels after hemorrhagic and ischemic stroke cannot be suppressed by dexamethasone indicating a dysregulation of the HPA axis in acute stroke (11, 12). Cortisol has direct effects on all immune cells as well as the body's inflammatory and immune response (12). It modulates the number, function, and differentiation of T-cells, monocytes-macrophages, eosinophils, mast cells, dendritic cells, and down-regulates cytokines and renders the host more susceptible to infection. Similarly, we observed a dysregulation of the HPA axis and delayed and diminished cytokine expression in diabetic mouse model of stroke (13), which prompted us to further investigate the immune-stress response in diabetic patients after a stroke.

In this prospective clinical study, we recruited patients presenting within 12 h of ischemic stroke onset and assessed their immune-stress during the hyper acute post stroke phase. To our knowledge this is the first study that targets the acute post-stroke stress immune response in diabetic stroke as a possible source for worse clinical outcome in this high risk population.

Materials and Methods

To analyze the stress-immune response in hyperglycemic compared to euglycemic acute stroke patients, we performed a prospective, institutional review board (IRB) approved clinical study in 24 adult patients. Patients presented with acute ischemic stroke, proven by computer tomography (CT), perfusion CT and/or brain magnetic resonance imaging (MRI) within 12h of symptom onset. To be eligible for enrollment, patients had to be free from

infections, had no previous stroke within the last year, and did not take any immunomodulatory drugs. Hyperglycemia was defined by an average blood glucose level of ≥150 mg/dl (≥8.3 mmol/l) measured on at least three blood samples (serum and/or finger stick glucose levels) collected during the first 24 h after stroke onset. Patients had serial blood draws at 0-12 h, 12-24 h, 24-48 h, 48-72 h and 72-96 h following symptom onset. Total white blood cell counts, neutrophils, hs-CRP, and blood cortisol were measured over time. Additionally HbA1c and glucose levels were measured at admission.

Peripheral circulating B-cells and T-cell subtypes (CD3, pan-T cells, CD4 helper T cells, CD8 suppressor T cells, CD19 B cells, and CD16+56 Natural Killer (NK) cells and activated neutrophils (CD45/ CD 63), activated monocytes (CD 45,CD 64/CD163) were measured over time by sequential flow cytometry. All laboratory results, neuroimaging and clinical data, utilizing National Institute of Neurological Disorders and Stroke data points, were abstracted into a RedCap database for statistical analysis. Flow data was analyzed using FlowJo software (Tree Star, Inc., USA).
 Clinical outcome measures included neurological status at the time of admission compared to discharge determined by the NIH stroke scale (NIH-SS), modified Rankling scale (mRS)at 90 days post stroke, discharge disposition (favorable: to home or post-acute rehabilitation or unfavorable: to long-term care facility or nursing home), in hospital morbidity and complications including symptomatic hemorrhagic transformation, cerebral edema and infection rate. Stroke volumes were measured on brain MRI using the ABC/2 method (14)

Statistical analysis

Descriptive statistics were performed in the form of geometric means and coefficients of variations for these biomarkers at each of the time points. Patient characteristics and outcomes in study groups were presented as percentages for categorical variables and the non-parametric. Categorical variables were compared using Fisher's exact test or the Chi-square test as associations as odd ratios. Continuous variables on the normality of distribution were compared using the t-test for independent samples. Multiple t-tests were performed between two study groups to compare the differences in clinical outcomes, neutrophil, lymphocytes, monocytes (Mean ± SEM). In order to analyze the relationship between clinical outcomes (NIH-stroke scale), cortisol level and neutrophil concentrations between two groups, Pearson's correlation analysis was performed. Tests of significance (Chi-square for categorical data), nonparametric tests and parametric tests were applied with a $P < 0.5$ being considered statistically significant. The p-values from the pairwise comparisons were adjusted for multiple comparisons using the Bonferroni correction. Data was analyzed using Graph Pad Prism 5.0 (Graph Pad Software Inc.) and presented as mean ±SEM.

Results

A total of 32 patients with acute ischemic stroke presenting within 12h of symptom onset were enrolled into our pilot study. Out of 32 subjects, 24 patients underwent serial blood draws, had complete data sets and were included in this analysis. Twelve of those patients (50%) were hyperglycemic during the first 24h of presentation (blood glucose level ≥8.3 mmol/l on three serial measurements) and 12 patients were euglycemic. Sixty percent of the hyperglycemic patients had known type-2 diabetes at the time of admission and an

additional 25% were subsequently diagnosed with type-2 diabetes or prediabetes during their hospital stay. Ninety-eight percent of patients were Caucasians. Baseline characteristics of study participants are presented in table 1, which also summarizes stroke subtypes, infarct volume, stroke complications and infection rates.

Table 1: Patient Characteristics

	Euglycemic Patients	**Hyperglycemic Patients**
Number of subjects	12	12
Age (Years) *(Mean ±SD)*	67.58 ± 12.74	68.5 ± 11.54
Gender *(% Female)*	41.7	50
HbA1C *(Mean ±SD)*	5.742 ± 0.614	6.991 ± 1.544 [*]
Average Glucose first 24h *(mg/dl)*	114 ± 7.44	156 ± 4.848 [*]
Average stroke volume	19.73 ± 14.38	97.90 ± 58.17 [*]
Stroke subtypes(%) *Large vessel distribution*	75	66
Lacunar infarct	8	17
Small vessel	17	17
Symptomatic hemorrhagic transformation *(%)*	17	42
Cerebral edema requiring treatment *(%)*	8.3	25
Infection requiring treatment *(%)*	17	42

*SD- Standard Deviation, and percentage may not sum to 100 because of rounding. *p < 0.05 Vs Euglycemic*

Table 1: Patient characteristics and clinical outcome data comparing hyperglycemic and euglycemic stroke patients. Age, gender and stroke severity at time of presentation were comparable in both groups. HbA1C, average blood glucose and stroke volume were significantly higher in hyperglycemic patients compared to euglycemic. Percentage of cerebral edema, hemorrhagic transformation and infection rates were higher in hyperglycemic patients compared to the euglycemic group. Values are expressed as mean ± SD. *p<0.05 vs euglycemic (n=12).

Euglycemic and hyperglycemic groups were comparable regarding age, gender and stroke subtypes. Hyperglycemic patients had higher HbA1c levels, larger stroke volumes (97.90 ± 58.17) and a higher rate of symptomatic hemorrhagic conversion (42%) compared to euglycemic individuals (19.73 ± 14.38, and 17% respectively).   In   addition, hyperglycemic individuals had a higher rate of malignant cerebral edema (25%) requiring osmotherapy and/or hemicranictomy, and had a higher infection rate (42 %) than euglycemic patients (17% and 8.3%, respectively), although these differences did not reach statistical significance.

Despite comparable admission NIH-SS in both groups, euglycemic patients had significantly better neurological function at time of discharge than their hyperglycemic counterparts (see table 2): Average differences in the NIH-SS at discharge were positive in euglycemic patients (+2.667 ± 3.5) and negative in hyperglycemic patients (-5.25 ± 8.38), indicating improvement of neurological function in the euglycemic group and worsening of neurological status in the hyperglycemic group. Furthermore, hyperglycemic patients were more likely to have a non-favorable discharge disposition (42%) than euglycemic patients (17%).

A total of 17 patients were evaluated at 90 days following discharge. Patients who were either deceased or discharged to nursing home were not included in the 90 day follow up data. The in hospital mortality rate was 8% and 25% in the euglycemic and hyperglycemic group, respectively. At 3 months follow-up, 43% of hyperglycemic patients remained disabled or dependent in their daily activities (mRS of 3-5) compared to only 12 % of their euglycemic counterparts (see table 2).

Table 2: Clinical outcomes at discharge and 90 days after stroke in patients

	Euglycemic Patients	Hyperglycemic Patients
Average NIH-SS score on admission (*Mean ±SD)*	**9.833 ± 7.9**	**9.333 ± 6.3**
Average NIH-SS score on discharge (*Mean ±SD)*	**7.818 ± 7.12**	**14.583 ± 9.56** [*]
Average NIH-SS score difference (*Admission-Discharge) Negative Value indicates worsening the outcome)*	**+ 2.667 ± 3.5**	**- 5.25 ± 8.38 ***
Favorable clinical outcome *Discharge to home or rehabilitation (%)*	**83**	**58**
Unfavorable clinical outcome *Discharge to acute long-term care facility or nursing home (%)*	**17**	**42**
mRS score at 90 days post stroke*(%)* ≥ 0-2 ≥ 3	**88** **12**	**57** **43**

*NIH-SS - National Institute of Health Stroke Scale, mRS- modified Rankin Scale, SD- Standard Deviation, and percentage may not sum to 100 because of rounding. *p < 0.05 Vs Euglycemic*

Table 2: Clinical outcomes in hyperglycemic and euglycemic stroke patients at admission, at hospital discharge and after 90 days post stroke. Negative values in differences of NIH-SS (National Institute of Health Stroke Scale) at admission compared to discharge indicate worsening of neurological function. Patients could be discharged home or to rehabilitation indicating more favorable outcome. NIH-SS score worsened in hyperglycemic compared to euglycemic stroke patients, and more favorable discharge disposition occurred in euglycemic than hyperglycemic patients (n=12). Modified Rankin Score (mRS) was obtained at 90 days of stroke. Values of mRS range from 0-6 (0 - most improvement, 6- death). A higher percentage of euglycemic patients scored between 0 to 2 indicating better recovery in euglycemic patients at 3 months than hyperglycemic patients. Values are expressed as mean ± SD. *p<0.05 vs euglycemic.

In addition, we systematically studied the acute stress-immune response following stroke in hyperglycemic and euglycemic patients: We observed a hyperacute immunosuppression following ischemic brain injury with significantly decreased B- and T-lymphocytes, including T-helper, T-suppressor and natural killer cells, and an increase in neutrophils and monocytes in all stroke patients. However, hyperglycemic patients showed stronger and longer lasting peripheral lymphocyte suppression, as well as significantly higher concentrations of circulating neutrophils and monocytes compared to euglycemic patients. Peripheral neutrophil activation and increase in monocytes post stroke was more pronounced and occurred earlier in the hyperglycemic than in the euglycemic group. The level of absolute neutrophils were significantly higher in hyperglycemic compared to euglycemic patients in the first 24 h and remained over the entire 96 h observation period (**Figure 1A**). However, activated neutrophils were higher in hyperglycemic patients but did not reach at statistical significance due to high variability in CD64 neutrophil number across both groups (data not shown). In addition, activated monocytes were significantly higher in hyperglycemic compared to euglycemic patients in the first 24 h and remained high over the observation period (**Figure 1B**). In hyperglycemic patients increased neutrophil concentrations were associated with a higher NIH-SS at discharge (r=-0.426, p<0.05) indicating worse neurological function (data not shown).

Hyperglycemic patients showed a stronger peripheral lymphocyte suppression than their euglycemic counterparts within the first 12h for B-cells and within 24h post stroke for T-lymphocytes (see **Figure 1C and 1D**). Greater depression of all subsets of T-cells was observed during first 24 h post stroke in hyperglycemic patients (see **Figure 2 A to 2C**).

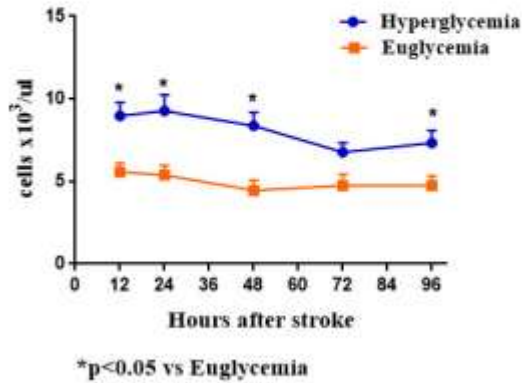Figure 1A: Post-stroke changes in neutrophils in euglycemic and hyperglycemic patients

*p<0.05 vs Euglycemia

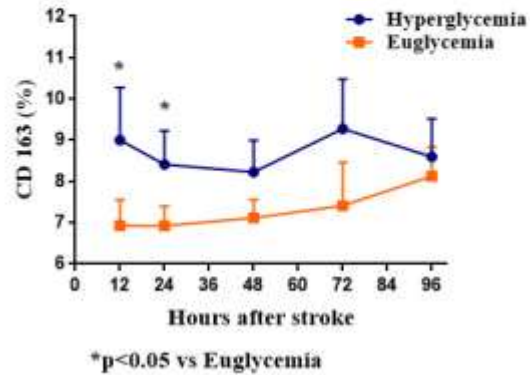Figure 1B: Post-stroke changes in monocytes in euglycemic and hyperglycemic patients

*p<0.05 vs Euglycemia

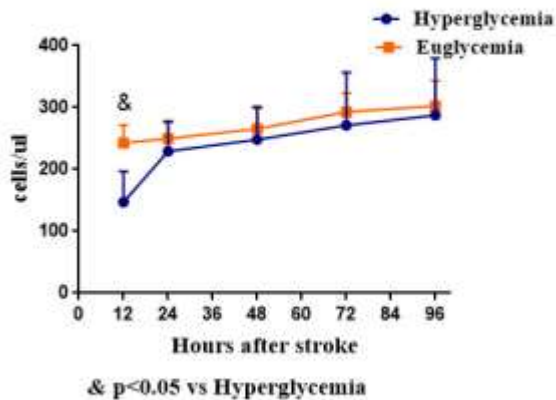Figure 1C: Post-stroke changes in B cells in euglycemic and hyperglycemic patients

& p<0.05 vs Hyperglycemia

Figure 1D : Post-stroke changes in T lymphocytes in euglycemic and hyperglycemic patients

& p<0.05 vs Hyperglycemia
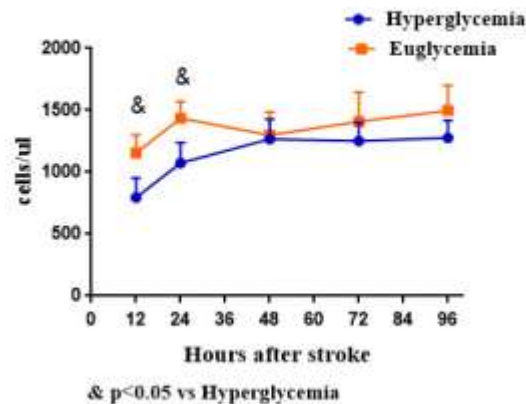
Figure 1: Dynamics of peripheral neutrophils, monocytes, T- and B-lymphocytes in blood samples of hyperglycemic and euglycemic patients at different time points after stroke. Patients had serial blood draws at 0-12 h 12-24 h, 24-48 h, 48-72 h and 72-96 h following stroke symptom onset. Absolute neutrophil were measured by central laboratory. T- lymphocytes, B-cell and activated monocyte concentrations were analyzed by sequential flow cytometry. **Fig. 1A** shows peripheral neutrophil concentrations (cells x 10^3/μl). Neutrophil numbers were significantly higher in hyperglycemic patients at 12 h, 24 h, 48 h and 96 h time points compared to the euglycemic group.*p<0.05 vs Euglycemia. **Fig 1B** shows a significant increase in expression of CD163, activated monocytes in hyperglycemic stroke patients within 24 h compared to euglycemic patients.* p<0.05 vs Euglycemia. **Fig. 1C** shows B-cells concentrations in hyperglycemic patients compared to euglycemic controls. There is a significant suppression of B-lymphocytes within the first 12h post stroke in hyperglycemic compared to euglycemic patients. $ p<0.05 vs Hyperglycemia. **Fig. 1D** shows the significant suppression T-lymphocytes concentration in hyperglycemic patients within the first 24 h compared to euglycemic. Results are expressed as mean ± SEM (n=12). & p<0.05 vs Hyperglycemia.

Figure 2A: Post-stroke changes in T Suppressor lymphocytes in euglycemic and hyperglycemic patients
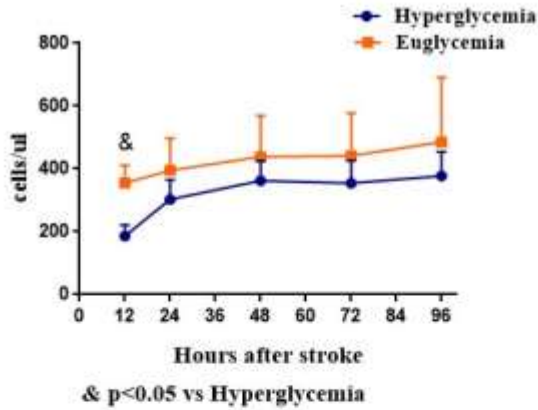

& p<0.05 vs Hyperglycemia

Figure 2B : Post-stroke changes in T helper lymphocytes in euglycemic and hyperglycemic patients

& p<0.05 vs Hyperglycemia
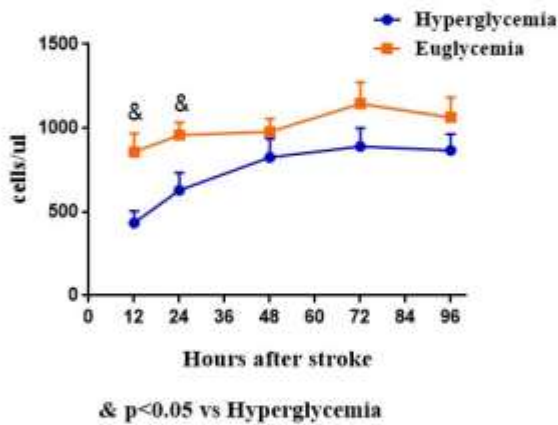
Figure 2C: Post-stroke changes in NK cells in euglycemic and hyperglycemic patients

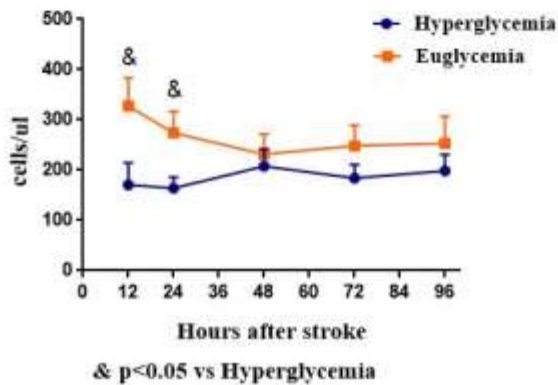

& p<0.05 vs Hyperglycemia

Figure 2: Post stroke changes in subpopulations of various T-lymphocytes in hyperglycemic and euglycemic stroke patients. **Fig. 2A** indicates higher levels of T-suppressor cells in euglycemic patients at all-time points and significantly higher concentrations within the first 12 h compared to the hyperglycemic group. **Fig. 2B** and **2C** show a similar pattern of T-helper cells and NK cells in euglycemic and hyperglycemic stroke patients. The concentration of both cell types were higher in euglycemic patients at all-time points, reaching significance at 12h and 24 h post stroke

In addition, cortisol levels were acutely elevated in most stroke patients. However it remained higher in hyperglycemic individuals throughout the observation period with significant differences between at 24h and 72 h post stroke (**Figure 3A**). Markers of inflammation, including the ESR (data not shown) and hs-CRP which are acute phase reactants, were significantly higher in hyperglycemic patients within the first 24 h after stroke and remained higher during the entire observation period compared to euglycemic individuals (**see Figure 3B**).

Figure 3A: Post-stroke changes in cortisol in euglycemic and hyperglycemic patients

*p<0.05 vs Euglycemia

Figure 3B: Post-stroke changes in hs-CRP in euglycemic and hyperglycemic patients
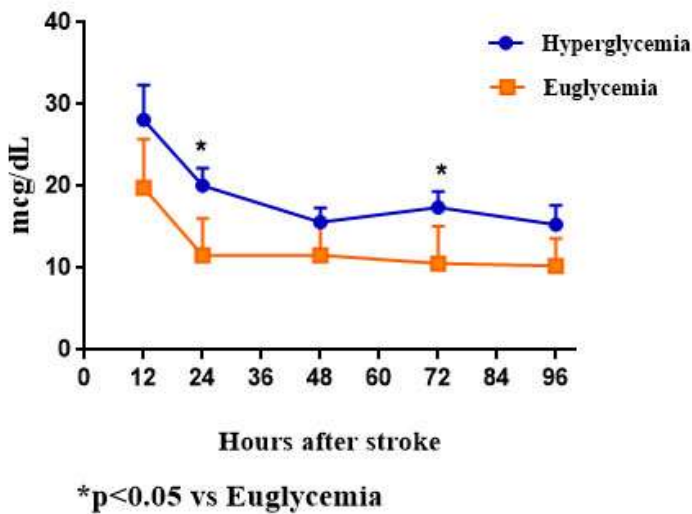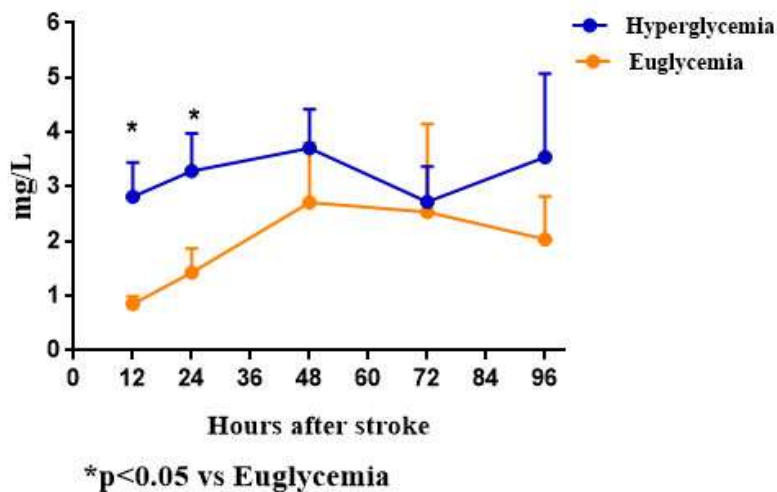
*p<0.05 vs Euglycemia

Figure 3A and 3B show higher concentrations of cortisol and high sensitive C-reactive protein (hs-CRP) in hyperglycemic individuals throughout the observation period compared to their euglycemic counterparts. The values were significantly higher in hyperglycemic group at 12h and 24h compared to the euglycemic group. Values are shown as mean ± SEM (n=12). *p<0.05 vs euglycemia.

Discussion

In this study we observed that hyperglycemic patients had larger stroke volumes and a higher rate of symptomatic hemorrhagic transformation and malignant cerebral edema, causing mass effect and acute neurological worsening, compared to euglycemic individuals which is consistent with previous studies (15, 16). Similar to others, we found that hyperglycemic patients had worse neurological outcome at the time of discharge and at 90 days post stroke (17) , and were more likely to have a non-favorable discharge disposition than their euglycemic counterparts, despite comparable NIH-SS admission scores. This difference in clinical outcomes was not confounded by use of thrombolysis with *i.v.* recombinant tissue plasminogen activator (t-PA) or thrombectomy (60 % patients received *i.v.* t-PA; treatment rate 60% in hyperglycemic group versus 50% in euglycemic group; one patient underwent thrombectomy after receiving *i.v.* t-PA) which could have resulted in improved recanalization rates and thus better neurological recovery. Also the differences were not explained by stroke subtype with both groups having comparable amounts of large vessel strokes.

Although this is a small clinical prospective pilot study, our findings suggest that alternative mechanisms may be responsible for the poor clinical outcome in diabetic and hyperglycemic stroke. In this study we found that stroke patients with elevated glucose levels had higher in-hospital infection rates, including urinary tract infection and pneumonia, than euglycemic patients (30% hyperglycemic versus 14% euglycemic group) which is consistent with an altered immune response in hyperglycemic patients rendering the host more susceptible to infections which exacerbates both, neurological deficits and mortality

It has been well established that there is activation of the innate and adaptive immune response following acute stroke (5). Multiple inflammatory cytokines, chemokines and molecules of danger associated patterns (DAMPS), cell adhesion molecules, proteases, prostanoids and leukotrienes are released into the bloodstream from the ischemic brain tissue and from the various cellular constituents of the immune system (18). These factors mediate inflammation and secondary brain injury, but also activate protective molecules and cell subtypes, such as M-2 monocytes, which are crucial for tissue remodeling and recovery (19) .

These stress-immune responses in the hyperglycemic state has to our knowledge not been previously studied in detail. It has been previously observed in general stroke populations that circulating neutrophils increase, peripheral lymphocytes decrease after stroke, and that there is impaired activation of cytokine production by T-cells and NK cells which correlates with stroke severity (20, 21). In our study we observed a similar post-stroke immune response that lasted up to 96 h after symptom onset, but this response was more pronounced in hyperglycemic compared to euglycemic patients, which is reflected in significantly higher circulating neutrophil concentrations and lower T-lymphocyte and B-cell levels within the acute post-stroke period in hyperglycemic compared to euglycemic individuals.

We also found that increased peripheral neutrophil levels were associated with worse neurological outcomes, suggesting an altered neutrophil function and/or neutrophils turnover in the hyperglycemic state following acute ischemic stroke. As part of the host defense, neutrophils form extracellular traps (NETs) by releasing cytotoxic proteins which kill pathogens, but

can also cause tissue injury (22). Neutrophils isolated from type-1 and type- 2 diabetic humans have been shown to be primed to produce extracellular traps (NETs), promoting tissue damage and impaired wound healing after injury (23, 24). It is possible that an increase in primed neutrophils in hyperglycemic stroke mediates enhanced brain injury by this mechanism (25).

Accumulating evidence suggests acute and long-lasting changes in the numbers and function of circulating lymphocytes and mononuclear cells which can be observed in both, animal models of ischemic stroke and stroke patients. In particular, the number of T- and B-lymphocytes decreases significantly after acute ischemic stroke. Such lymphopenia can last for months with a gradual recovery over time (20, 26).

Similar to previous studies we found elevated cortisol levels within 24 h after stroke in the majority of our patients (12). However, cortisol levels were higher in hyperglycemic than in euglycemic patients throughout the entire 96 h observation period in the present study. The higher levels of cortisol in hyperglycemic patients could lead to a suppressed innate and adaptive immune response resulting in a higher infection rate, and worse clinical outcome compared to euglycemic patients.

Stress activates the neuroendocrine pathway to release catecholamines, glucocorticoids and cytokines, which have profound influence on immunity in both, humans and rodents. Normally, the initial stress-induced activation of the hypothalamus is followed by cortisol-induced feedback suppression of ACTH levels and increased responsiveness of the adrenal glands (27). Our preliminary clinical data suggest that this regulation is dysfunctional in hyperglycemic patients.

Glucocorticoids cause neutrophilia either by recruitment of neutrophils from the bone marrow or has mixed effects on neutrophil apoptosis and subsequent tissue fate (28). It can induce neutrophil apoptosis and inhibition of neutrophil extravasation into the infarcted area, downregulating the extent of inflammation (29). On the other hand, cortisol can increase anti-apoptotic factors and decrease pro-apoptotic molecules leading to prolonged neutrophil survival and upregulation of the inflammatory response. Following ischemic stroke we have previously observed significantly elevated cortisol and neutrophil levels in diabetic mice and in diabetic patients compared to their nondiabetic counterparts suggesting a shift towards reduced neutrophil apoptosis, increased tissue inflammation and impaired glucocorticoid receptor signaling in diabetic stroke (manuscripts under review).

In line with these observations, we observed significantly higher concentrations of hs-CRP in hyperglycemic compared to euglycemic stroke patients. Preclinical and clinical studies suggest that hs-CRP has many pathophysiological roles in the inflammatory process (30) Elevated serum levels of hs-CRP are not disease specific, but hs-CRP is a sensitive marker of the inflammatory response associated with tissue injury, infectious agents, and immunologic stimuli (31). Higher levels of hs-CRP in hyperglycemic compared to euglycemic stroke patients in our study may reflect an altered systemic inflammatory response following ischemic stroke, possibly superimposed on a chronic pro-inflammatory state in diabetes (32), or may be associated with more extensive tissue injury and a higher infection rate observed in the hyperglycemic group of the present study.

The higher rates of malignant cerebral edema and hemorrhagic stroke transformation in hyperglycemic compared to euglycemic

stroke patients which was observed on our study confirms previous study results (15), and suggests that the blood-brain barrier in hyperglycemic individuals is more compromised than in euglycemic individuals after acute stroke. This could be due to an increased expression of vascular cell adhesion molecules in the hyperglycemic state causing enhanced endothelial neutrophil adhesion and transmigration to the side of injury with subsequent more pronounced inflammation and tissue damage (33).

Our study has several limitations. The data came from one center and from a mainly Caucasian population and thus the results may this not be generalizable. Additionally, due to the limited number of patients the findings of this study would need to be confirmed within a larger cohort. Lastly, the study shows associations between various circulating immune cells, cortisol levels and clinical outcome, but does not study possible causation or underlying cellular mechanisms.

Conclusion

Based on our knowledge this is the first study to systematically investigate the acute stress-immune response in acute hyperglycemic stroke patient compared to euglycemic. Our results indicate that the stress-immune response after stroke is altered in patients with hyperglycemia and diabetes, and that this altered response is associated with worse clinical outcome. Furthermore this study suggests that neutrophils play a key role for stroke outcome in the hyperglycemic state and modulation of neutrophil function may thus help to improve outcomes for this this high risk stroke population. Future studies will attempt to explore underlying pathophysiology and therapeutic strategy for this high risk patient population.

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