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Neeraj Badjatia

Serge Cremers

Jan Claassen

E S. Connolly

Stephan A. Mayer
Henry Ford Health System

See next page for additional authors

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ARTICLE

Serum glutamine and hospital-acquired infections after aneurysmal subarachnoid hemorrhage

Neeraj Badjatia, MD, MS, Serge Cremers, MD, Jan Claassen, MD, E. Sander Connolly, MD, Stephan A. Mayer, MD, Wahida Karmally, DrPH, RD, and David Seres, MD, ScM, PNS FASPEN

Correspondence
Dr. Badjatia
nbadjatia@umm.edu

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Abstract

Objective

To understand nutritional and inflammatory factors contributing to serum glutamine levels and their relationship to hospital-acquired infections (HAIs) after aneurysmal subarachnoid hemorrhage (SAH).

Methods

A prospective observational study of patients with SAH who had measurements of daily caloric intake and C-reactive protein, transthyretin, tumor necrosis factor α receptor 1a (TNFαR1a), glutamine, and nitrogen balance performed within 4 preset time periods during the 14 days after SAH. Factors associated with glutamine levels and HAIs were analyzed with multivariable regression. HAIs were tracked daily for time-to-event analyses. Outcome 3 months after SAH was assessed by the Telephone Interview for Cognitive Status and modified Rankin Scale.

Results

There were 77 patients with an average age of 55 ± 15 years. HAIs developed in 18 (23%) on mean SAH day 8 ± 3. In a multivariable linear regression model, negative nitrogen balance (p = 0.02) and elevated TNFαR1a (p = 0.04) were independently associated with higher glutamine levels during the study period. The 14-day mean glutamine levels were lower in patients who developed HAI (166 ± 110 vs 236 ± 81 μg/mL, p = 0.004). Poor admission Hunt and Hess grade (p = 0.04) and lower glutamine levels (p = 0.02) predicted time to first HAI. Low 14-day mean levels of glutamine were associated with a poor recovery on the Telephone Interview for Cognitive Status score (p = 0.03) and modified Rankin Scale score (p = 0.04) at 3 months after injury.

Conclusions

Declining glutamine levels in the first 14 days after SAH are influenced by inflammation and associated with an increased risk of HAI.

From the Section of Neurocritical Care (N.B.), Program in Trauma, Department of Neurology, University of Maryland School of Medicine, Baltimore; Neurological Institute of New York (J.C., E.S.C.), NY; Department of Neurology (S.A.M., W.K.), Henry Ford Hospital, Detroit, MI; and Institute of Human Nutrition and Division of Preventive Medicine and Nutrition (S.C., W.K.), Department of Internal Medicine (D.S.), Columbia University College of Physicians and Surgeons, New York, NY.

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Glossary

CI = confidence interval; **HAI** = hospital-acquired infection; **IQR** = interquartile range; **SAH** = subarachnoid hemorrhage; **TDFHA** = tridecafluoroheptanoic acid; **TICS** = Telephone Interview for Cognitive Status; **TNFαR1a** = tumor necrosis factor α receptor 1a.

Malnutrition has been associated with impaired immunologic function leading to increased rates of infection.[1] An assessment of nutritional profiles measured by indirect calorimetry found that patients with subarachnoid hemorrhage (SAH) had average resting energy expenditure rates between 40% and 75% above baseline levels.[2,3] We have described an independent relationship between inflammation and negative nitrogen balance that was predictive of hospital-acquired infection (HAI) and long-term recovery after SAH.[4] As a further exploration of the relationship between protein catabolism and secondary injury after SAH, we hypothesized that glutamine levels may have a significant role in identifying patients at risk of developing secondary complications with a rapid decline correlated to the development of HAIs after SAH.

In non-SAH populations, a net negative energy balance has been shown to result in protein catabolism and depletion of amino acids necessary for cellular repair and host defenses. Clinically this has been manifest by low levels of amino acids such as glutamine.[5] None of the previous studies have investigated the relationships among serum glutamine levels, metabolic state, and outcome after SAH, where the stress responses after SAH are similar to that observed in medical illnesses such as acute respiratory distress syndrome and sepsis.[2] We investigated this relationship by analyzing levels of serum glutamine and tumor necrosis factor α receptor 1a (TNFαR1a) and time to HAI in a subset of patients previously enrolled in a prospective observational cohort study of the sequelae of the immune-mediated malnutrition after SAH.

Methods

Patient selection and data collection

This is a retrospective analysis of a subset of consecutively enrolled patients in a prospective observational study that had available serum from all 4 predefined phases during the study period[4] and serum collected for amino acid and inflammatory marker analysis. Clinical care for patients with SAH has been described previously[6] and conformed to established guidelines.[7,8] All underwent serial assessments of inflammatory and metabolic parameters during the first 14 days after SAH in a systematic manner as previously reported.[4] Inflammatory and metabolic parameters were measured during the same 24 hours within each period. Data collection was considered complete in instances when patients died or were discharged from the hospital prior to completion of the study period. The SAH data collection materials and practices used in the ongoing SAH outcomes project have been previously described.[4] All were tested at 3 months for functional disability. Each patient was screened daily for the development of infectious complications, using established criteria for HAIs.[9] We recorded the calendar date for each infectious complication, which allowed for the quantification of the true incidence of HAIs as those infections that developed ≥72 hours after ictus and for time-to-event analysis.[10]

Biomarker measurements

Quantitation of glutamine

Glutamine was measured in plasma samples using ultra-performance liquid chromatography–tandem mass spectrometry. Glutamine was assayed in the plasma samples by mixing 10 μL of plasma and 10 μL of internal standard with 1 mL of TDFHA (tridecafluoroheptanoic acid) in an LCMS (liquid chromatography–mass spectrometry) vial. After vortexing for 5 minutes, the vial was placed in an autosampler at 4°C.

Liquid chromatography–tandem mass spectrometry analysis was performed on a platform comprising a triple quadrupole Waters Xevo TQ-S (Waters, Milford, MA) equipped with an electrospray ionization source and integrated with a Waters Acquity UHPLC (ultra-high performance liquid chromatography) controlled by MassLynx software 4.1. Chromatographic separation was performed by injecting 5 μL of the sample onto a Waters C18 BEH column (2.1 × 100 mm, 1.7 μm, 130 Å) equipped with a Vanguard BEHC18 precolumn and maintained at 35°C. The flow rate was maintained at 650 μL/min. The initial flow conditions were 99.5% solvent A (water containing 0.5 mm TDFHA) and 0.5% solvent B (0.5 mm TDFHA in acetonitrile). Solvent B was raised to 30% over 14 minutes and lowered back to 0.5% by 17.5 minutes and remained at initial conditions for a total run time of 31.5 minutes. The retention time for glutamine was 2.02 minutes. The mass spectrometer was operated under multiple reaction monitoring mode with positive electrospray ionization with the following parameters: capillary voltage: 1.5 kV; cone gas flow: 300 L/h; desolvation gas: 1,200 L/h; and gas temperature: 600°C. The multiple reaction monitoring transition 146.9 > 83.9 was utilized for quantitation with cone voltage 25 and collision energy 16. Peak integration and data analysis were performed with TargetLynx 4.1 (Waters). Intra- and interassay precision was <10%.

Quantitation of TNFαR1a

TNFαR1a was measured in plasma by Quantikine Human sTNFR1a immunoassay (R&D Systems, Minneapolis, MN).

Intra- and interassay precision was <5.0% and <8.8%, respectively. Normal range is 484–1,407 pg/mL.

Outcome assessments

Outcome was assessed prospectively 3 months post-hemorrhage with a 7-point version of the modified Rankin Scale rated from death to symptom-free full recovery[11] and the Telephone Interview for Cognitive Status (TICS).[12] All clinical and outcome endpoints were classified according to a priori criteria and adjudicated at a weekly research meeting, as previously described.[4]

Statistical analysis

Given this was a retrospective analysis, we did not perform a sample size calculation a priori. The sample size was based on the last 77 consecutive subjects enrolled in the previous cohort study. Continuous variables were assessed for normality and reported using accepted standards for parametric and nonparametric data. Categorical variables were reported as count and proportions in each group. Low 14-day mean serum glutamine levels were defined as values below the 14-day mean glutamine level. Multivariable linear regression analyses were performed to determine factors associated with serum glutamine levels by entering in those factors found to have a p value ≤0.1 on univariate analysis. The occurrence of the first HAI was treated as a censored event by postbleed day and corresponding study period. Baseline characteristics that were found on univariate analysis to be associated ($p \leq 0.1$) with HAI were entered into a Cox proportional hazards model to calculate hazard ratios and corresponding 95% confidence interval (CI) for developing HAI. Tests for interaction were performed and reported when found to be significant. For all tests, significance was set at $p < 0.05$. All analyses were performed with SPSS version 24.0 (IBM Corp., Armonk, NY).

Table 1 Baseline characteristics of patients with subarachnoid hemorrhage

	Hospital-acquired infection		
Admission characteristics	**No (n = 59)**	**Yes (n = 18)**	***p* Value**
Age, y, mean (SD)	54 (13)	58 (12)	0.13
Women, n (%)	37 (63)	14 (78)	0.27
Body mass index, kg/m^2	30 (7)	34 (11)	0.04
Medical history, n (%)			
Hypertension	23 (39)	10 (56)	0.21
Diabetes mellitus	3 (5)	2 (11)	0.33
Ethnicity, n (%)			0.43
Black	14 (24)	4 (22)	
White, non-Hispanic	21 (36)	5 (28)	
White, Hispanic	22 (37)	7 (39)	
Asian	2 (3)	2 (11)	
Aneurysm clipping, n (%)	40 (68)	11 (61)	0.6
APACHE II score	12 (7)	18 (8)	0.002
Hunt and Hess grade, n (%)			0.004
1 and 2: headache	35 (59)	5 (29)	
3: stupor	12 (20)	3 (17)	
4: obtunded	8 (14)	7 (39)	
5: Coma	4 (7)	3 (17)	
Modified Fisher score, n (%)			0.43
1: thin clot	16 (27)	3 (17)	
2: thin clot and IVH	0 (0)	1 (6)	
3: thick clot	32 (54)	5 (28)	
4: thick clot and IVH	11 (19)	9 (50)	

Abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II; IVH = intraventricular hemorrhage.

Standard protocol approvals, registrations, and patient consents

Consent and conduct of this study were approved by the institutional review board and consistent with guiding principles for research involving humans.[13]

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Patient characteristics

There were 77 patients that underwent serum glutamine and TNFαR1a analysis, with a mean age of 55 ± 15 years, 66% women, a median admission Hunt and Hess grade of 2, and modified Fisher score of 3. During the first 14 days after hemorrhage, 18 patients (23%) developed an HAI. Baseline comparisons of patients by HAI status are shown in table 1. Pneumonia was the most common infection, occurring in 15 patients (19%), followed by urinary tract infections (n = 11, 15%), meningitis (n = 6, 8%), and blood stream infections (n = 2, 3%).

Factors associated with serum glutamine levels

The mean serum TNFαR1a level during the study period was 1,228.25 ± 606.6 pg/mL and glutamine 219.4 ± 94 μg/mL. There was a progressive decline in the serum glutamine levels over the 14-day study period (analysis of variance *F* test, $p < 0.001$) In a multivariate linear regression model adjusting for admission Hunt and Hess grade, modified Fisher score, and caloric intake, serum glutamine levels were found to be associated with a net negative nitrogen balance (β: 5.023; 95% CI: 0.806–9.241, $p = 0.02$) and TNFαR1a levels (β: −0.036; 95% CI: −0.07 to −0.002, $p = 0.04$).

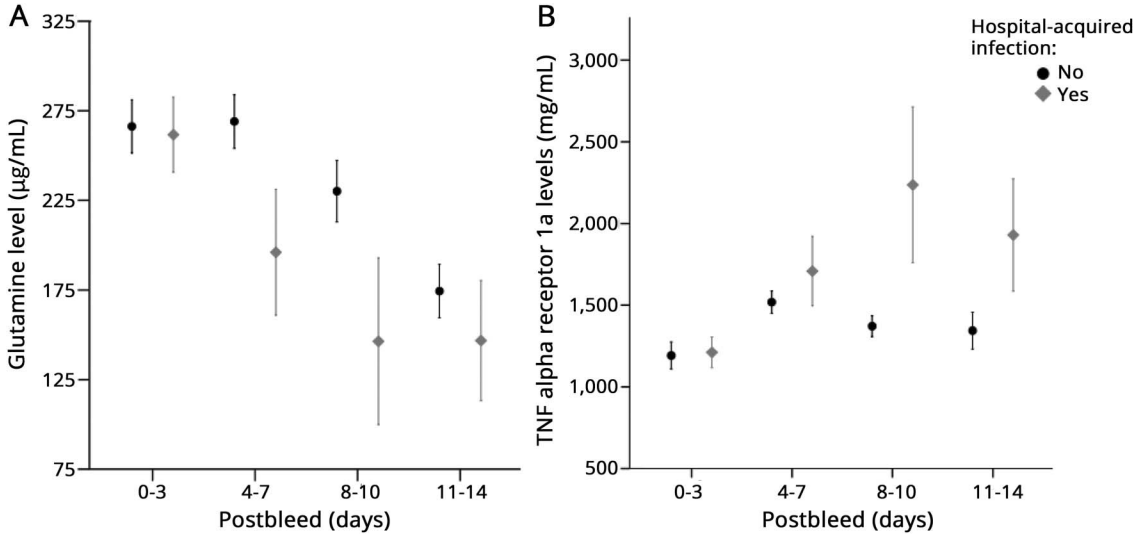
Outcome assessments

HAI developed on postbleed day 8 ± 3. Patients developing HAI had lower serum glutamine levels (232 ± 83 vs 163 ± 112 μg/mL, $p = 0.01$) and higher TNFαR1a levels (1,503 ± 543 vs 1,138 ± 611 pg/mL, $p = 0.03$). A graphical representation of serum glutamine and TNFαR1a levels over the study period by HAI status is shown in figure 1. In a univariate model, a low mean serum glutamine level (<219 μg/mL) was associated with time to development of an HAI by postbleed day 14 (figure 2). Low 14-day mean serum glutamine level as well as admission Hunt and Hess grade were associated with time to development of HAI after correcting for caloric intake, age, mechanical ventilation, and body mass index (table 2). The median (25th %ile, 75th %ile) 3-month modified Rankin Scale score was 2 (1, 4), with a 12% (9/77) mortality rate. On univariate analysis, patients with a low mean 14-day serum glutamine level were associated with a lower median TICS score (28; interquartile range [IQR]: 15] vs 33 [IQR: 7], $p = 0.03$) and higher median modified Rankin Scale score (3 [IQR: 3] vs 2 [IQR: 3], $p = 0.04$) at 3 months after injury. In separate multivariable models adjusting for age, Hunt and Hess grade, and occurrence of delayed cerebral ischemia, a low mean 14-day serum glutamine level was not associated with a lower modified Rankin Scale score or TICS score.

Discussion

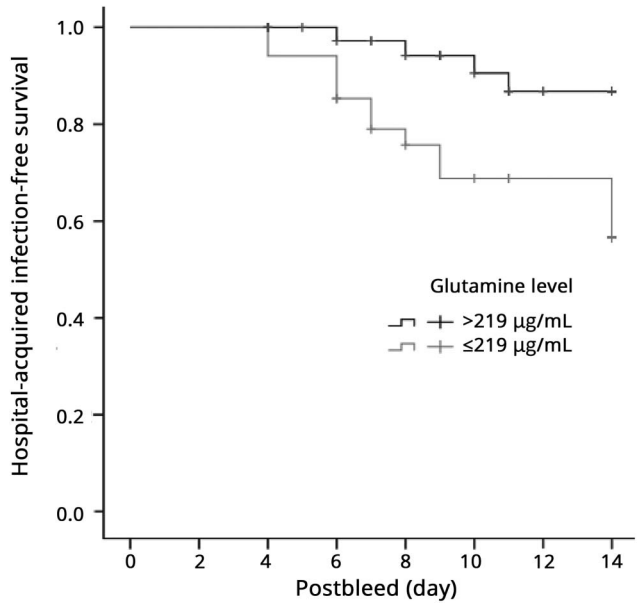
We found that rapidly declining serum glutamine that was linked to net negative nitrogen balance and inflammation was

Figure 1 Relationship between serum glutamine TNFαR1a levels and HAIs



(A) Difference between serum glutamine levels in those who developed HAI vs those who did not develop HAI. (B) Difference between serum TNFαR1a levels in those who developed HAI vs those who did not develop HAI. HAI = hospital-acquired infection; TNF = tumor necrosis factor; TNFαR1a = TNF-α receptor 1a.

Figure 2 Time to hospital-acquired infections



associated with a significantly shorter time to development of HAI within the first 14 days after SAH. Furthermore, on univariate analysis, low 14-day mean serum glutamine levels were associated with poor cognitive and functional recovery 3 months after injury.

Similar findings with protein energy catabolism leading to glutamine depletion and HAIs have been identified in non-SAH populations.[5] Glutamine serves as a vital cell-signaling molecule in states of illness and injury[14] and regulate the expression of many genes related to metabolism, signal transduction, cell defense, and repair, and to activate intracellular signaling pathways.[15] The release of glutamine from muscle and other sources after stress, illness, and injury serves as a "stress signal," which results in activation of genes vital to cellular protection and immune regulation.[16] The presence of adequate circulating glutamine is likely an important factor in preserving cellular energetics, preservation of muscle mass, and supporting immune function during critical illness.

This study has limitations worth noting. The patients in this retrospective analysis are a subset of a previous prospective study, and as a result, our conclusions are limited by the post hoc analysis. As such, we cannot eliminate the possibility that TNFαR1a rose in response to infection, and although our analyses do indicate a strong relationship between glutamine and TNFαR1a, we cannot prove causality in this pilot study. However, the rates of infection are similar to our larger cohort study, and by selecting consecutive patients without regard to their outcomes, we believe any selection bias was minimized. In addition, our results may be inadequately powered, although they do provide preliminary evidence for a link between declining serum glutamine levels and infectious complications after SAH. Finally, as we previously noted,[4] our outcome measures of TICS and modified Rankin Scale may not be appropriate measures of recovery related to malnutrition. Preserved motor strength, physical recovery, and fatigue are likely better markers of the true influence of immune-mediated malnutrition on outcome after critical illness.[17]

A composite view of our results from this and previous studies indicates that malnutrition, related to hypermetabolism, underfeeding, and inflammation-mediated protein catabolism is prevalent after SAH and associated with short-term secondary injury and long-term poor outcome.[4,18–20] Aspects related to undernutrition may be modifiable, but recent studies indicate that the overall amount of caloric delivery may not be as important as specific substrate delivery.[21,22] Protein energy malnutrition may be a suitable target for intervention with amino acid supplementation focusing on a reduction of infectious complications and improving recovery. Alternatively, the decrement in glutamine may represent epiphenomenon of inflammatory disease and may only signal risk.

One aspect of malnutrition-related injury and recovery not addressed by current studies involves the influence of acute muscle wasting on physical recovery and fatigue after SAH. Muscle wasting is directly linked to a catabolic state, and recent intervention studies have demonstrated an ability to reduce muscle wasting with a targeted nutritional supplementation and exercise regimen.[23] A dual-therapy approach may be a reasonable next step to better understand the implications of malnutrition and methods by which to optimize physical recovery after SAH.

Author contributions

Dr. Badjatia: drafting/revising the manuscript for content, including medical writing for content, study concept or design, analysis or interpretation of data, study supervision or coordination. Dr. Cremers: drafting/revising the manuscript for content, including medical writing for content, analysis or interpretation of data. Dr. Claassen: drafting/revising the manuscript for content, including medical writing for content.

Table 2 Predictors of hospital-acquired infection in the first 14 days after subarachnoid hemorrhage

	Hazard ratio	95% confidence interval	*p* Value
Hunt and Hess grade	1.426	1.026–1.982	0.04
Glutamine level	0.994	0.989–0.999	0.02

Dr. Connolly: drafting/revising the manuscript for content, including medical writing for content. Dr. Mayer: drafting/revising the manuscript for content, including medical writing for content, analysis or interpretation of data. Dr. Karmally: drafting/revising the manuscript for content, including medical writing for content, acquisition of data, study supervision or coordination. Dr. Seres: drafting/revising the manuscript for content, including medical writing for content, analysis or interpretation of data, study supervision or coordination.

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Disclosure

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