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Serum glutamine and hospital-acquired infections after aneurysmal subarachnoid hemorrhage

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Neurology® 2018;91:e421-e426. doi:10.1212/WNL.0000000000005902

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.
Malnutrition has been associated with impaired immunologic function leading to increased rates of infection. 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. 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. 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. 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. We investigated this relationship by analyzing levels of serum glutamine and tumor necrosis factor 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.

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 (tridecafluorooctanoic 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 UPLC (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 pre-column 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). Intrarand 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. All clinical and outcome endpoints were classified according to a priori criteria and adjudicated at a weekly research meeting, as previously described.

**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 ≤ 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

<table>
<thead>
<tr>
<th>Admission characteristics</th>
<th>Hospital-acquired infection</th>
<th></th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n = 59)</td>
<td>Yes (n = 18)</td>
<td></td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>54 (13)</td>
<td>58 (12)</td>
<td>0.13</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>37 (63)</td>
<td>14 (78)</td>
<td>0.27</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30 (7)</td>
<td>34 (11)</td>
<td>0.04</td>
</tr>
<tr>
<td>Medical history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>23 (39)</td>
<td>10 (56)</td>
<td>0.21</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (5)</td>
<td>2 (11)</td>
<td>0.33</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>Black</td>
<td>14 (24)</td>
<td>4 (22)</td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>21 (36)</td>
<td>5 (28)</td>
<td></td>
</tr>
<tr>
<td>White, Hispanic</td>
<td>22 (37)</td>
<td>7 (39)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2 (3)</td>
<td>2 (11)</td>
<td></td>
</tr>
<tr>
<td>Aneurysm clipping, n (%)</td>
<td>40 (68)</td>
<td>11 (61)</td>
<td>0.6</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>12 (7)</td>
<td>18 (8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hunt and Hess grade, n (%)</td>
<td></td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>1 and 2: headache</td>
<td>35 (59)</td>
<td>5 (29)</td>
<td></td>
</tr>
<tr>
<td>3: stupor</td>
<td>12 (20)</td>
<td>3 (17)</td>
<td></td>
</tr>
<tr>
<td>4: obtunded</td>
<td>8 (14)</td>
<td>7 (39)</td>
<td></td>
</tr>
<tr>
<td>5: Coma</td>
<td>4 (7)</td>
<td>3 (17)</td>
<td></td>
</tr>
<tr>
<td>Modified Fisher score, n (%)</td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>1: thin clot</td>
<td>16 (27)</td>
<td>3 (17)</td>
<td></td>
</tr>
<tr>
<td>2: thin clot and IVH</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td></td>
</tr>
<tr>
<td>3: thick clot</td>
<td>32 (54)</td>
<td>5 (28)</td>
<td></td>
</tr>
<tr>
<td>4: thick clot and IVH</td>
<td>11 (19)</td>
<td>9 (50)</td>
<td></td>
</tr>
</tbody>
</table>

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 TNFaR1a 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 TNFaR1a 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 TNFaR1a 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 TNFaR1a levels (1,503 ± 543 vs 1,138 ± 611 pg/mL, p = 0.03). A graphical representation of serum glutamine and TNFaR1a 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 TNFaR1a levels and HAI

(A) Difference between serum glutamine levels in those who developed HAI vs those who did not develop HAI. (B) Difference between serum TNFaR1a levels in those who developed HAI vs those who did not develop HAI. HAI = hospital-acquired infection; TNF = tumor necrosis factor; TNFaR1a = TNF-α receptor 1a.
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. Glutamine serves as a vital cell-signaling molecule in states of illness and injury and regulate the expression of many genes related to metabolism, signal transduction, cell defense, and repair, and to activate intracellular signaling pathways. 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. 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 TNFaR1a rose in response to infection, and although our analyses do indicate a strong relationship between glutamine and TNFaR1a, 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, 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.

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. 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. 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. 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

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Hazard ratio</th>
<th>95% confidence interval</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunt and Hess grade</td>
<td>1.426</td>
<td>1.026–1.982</td>
<td>0.04</td>
</tr>
<tr>
<td>Glutamine level</td>
<td>0.994</td>
<td>0.989–0.999</td>
<td>0.02</td>
</tr>
</tbody>
</table>
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.

Study funding
Supported by the National Center for Advancing Translational Sciences, NIH, through grant KL2 TR000081, formerly the National Center for Research Resources, grant KL2 RR024157. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosure
N. Badjatia reports research funding support from VA Maryland Exercise and Robotics Center of Excellence and from the Maryland Industrial Partnerships Program (MIPS 6021). S. Cremers, J. Claassen, and E. Connolly report no disclosures relevant to the manuscript. S. Mayer reports consulting income from Edge Therapeutics and Actelion Pharmaceuticals (now Idorsia Pharmaceuticals). W. Karmally and D. Seres report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Received October 18, 2017. Accepted in final form April 27, 2018.

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