Plasma Glial Fibrillary Acidic Protein Levels in a Child with Sickle Cell Disease and Stroke

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Key Words

Chronic transfusion • Glial fibrillary acidic protein • Sickle cell • Stroke

Abstract

A 12-year-old boy with HbSS sickle cell disease (SCD) was admitted with an acute febrile illness and developed overt stroke 3 days later. Plasma glial fibrillary acidic protein levels were elevated, as compared to pediatric controls, 32 h prior to the clinical diagnosis of stroke, peaked immediately prior to the exchange transfusion, and remained elevated 1 year later despite chronic transfusion therapy. Stroke in SCD can occur in the setting of acute illness, and a biomarker that could predict the onset and triage ill children to therapeutic intervention more quickly would be useful.

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Introduction

Overt stroke occurs in approximately 10% of children with sickle cell disease (SCD) [1]. The cause of stroke in SCD is multifactorial and involves hemolysis, endothelial dysfunction, chronic inflammation, hypercoagulability, and altered adherent properties of sickled red blood cells

[2]. Management of acute stroke includes emergent red cell exchange followed by chronic red cell transfusion indefinitely [3, 4]. Glial fibrillary acidic protein (GFAP) is a brain-specific cytoskeletal protein that is a well-studied biomarker of brain injury in animal models and clinical settings of brain injury, including acute stroke in adults [5–8]. To our knowledge, using a blood biomarker to predict, diagnose, or track stroke outcomes in SCD has not been reported. In an ongoing proteomic study, we observed that GFAP was detectable in cross sectional plasma samples from clinically well children with SCD. We hypothesized that longitudinal plasma levels of GFAP in a child with SCD and acute stroke would detect ongoing brain injury.

Methods

GFAP was measured using an electrochemiluminescent immunoassay (MesoScale Discovery) that is based on the assay of Petzold et al. [9]. Briefly, GFAP was measured in undiluted duplicate plasma samples using the monoclonal anti-GFAP blend SMI-26 (Covance, Princeton, N.J., USA). GFAP at 100 ng in 30 μ l PBS per well was incubated overnight in standard-bind MSD plates for capture. Polyclonal anti-GFAP (Dako, Carpinteria, Calif., USA) directly conjugated with Sulfo-Tag (MesoScale Discovery) was used for detection at 1 μ g/ml in PBS. Standard curves were made with 4-fold dilutions of purified GFAP (Calbiochem, La Jolla,

Fig. 1. Neuroimaging of acute stroke in a 12-year-old HbSS patient who had plasma GFAP followed serially. **a** Noncontrast head CT immediately after clinical diagnosis of stroke showing a wedge of hypoattenuation in the right frontal lobe (arrow). **b** FLAIR MRI image 29 h after clinical diagnosis of stroke showing an evolving right anterior cerebral artery infarct (arrow).

Calif., USA) in 1% bovine serum albumin (SeraCare Life Sciences, Milford, Mass., USA). Plasma samples were obtained from discard blood procured as part of an IRB-approved research study with an informed consent exemption. Samples were frozen at -80°C until assayed. Sixty children, aged 5–16 years, were selected as pediatric controls. Healthy controls were free of acute illness and any chronic illness other than obesity, asthma, and behavior disorders. Stability of GFAP at room temperature was demonstrated for 48 h with a CV of 2.5% at 2 ng/ml and 12.1% at 0.2 ng/ml.

Case Report

A 12-year-old, right-handed boy with HbSS SCD was admitted for vomiting, diarrhea, chest pain, fever to 39°C , and hypoxemia. He was initially managed with oxygen and intravenous fluids, morphine, ketorolac, and ceftriaxone. Serial chest X-rays showed no pulmonary infiltrate, and blood, stool, and urine cultures were negative. Oral oxycodone was substituted for IV morphine, and he was weaned off oxygen. The patient improved clinically and was preparing on hospital day 3 for discharge the following day, but in the night of hospital day 3 he became confused, and on hospital day 4 the patient was noted to have a fever to 40°C , be uncommunicative with his parents, and have mild left arm and leg weakness. A CT of the head was performed which showed a large wedge-shaped right frontal infarct with a mild mass effect. Manual red blood cell exchange was performed emergently, with a reduction in HbS from 80.3 to 29.8%. Ceftriaxone was increased to doses appropriate for meningitis, and vancomycin was started. He was subsequently noted to be minimally verbal, incontinent of urine and stool, and hemiplegic on the left side. MRI images 29 h after the diagnosis of stroke confirmed a right frontal lobe infarct in the ACA distribution (fig. 1). Multiple T2 and FLAIR hyperin-

tense lesions that did not show restricted diffusion were present in the left centrum semiovale and frontal white matter, indicating prior cerebral infarcts that were not previously apparent clinically. CSF examination showed 6 WBC/ μl and 2 RBC/ μl , and CSF cultures were negative. Neurologic improvement was noted after the first red cell exchange, but the patient became aphasic 48 h after his initial stroke, and CT and subsequently MRI confirmed a new left operculum infarct (not shown). A second manual red cell exchange was performed with a final HbS of 13.9%. The patient was discharged to an inpatient rehabilitation hospital 8 days after the initial stroke and received monthly red blood cell transfusions to maintain an HbS $<30\%$. Sixteen weeks after the initial stroke, the patient underwent a pial synangiosis procedure for severe moyamoya disease combined with the history of stroke. In 1 year of follow-up from the initial stroke, there have been no additional neurologic events. Brain MRI performed at 4, 6, and 12 months after the stroke showed no new ischemic changes. A transcranial Doppler ultrasound had been performed at 8 years of age that demonstrated normal timed average mean velocities in the middle cerebral arteries (159 cm/s left and 153 cm/s right) indicating no increased risk of stroke at that time.

GFAP Levels in Controls and in a Patient with Overt Stroke

The 95th percentile cutoff of GFAP among pediatric controls was 0.227 ng/ml. The patient's sibling, who is matched for disease but not neurologic injury, also served as a control. One week after the stroke in the proband, the patient's sister, an 8 year old also with homozygous SCD, developed a febrile illness without neurologic sequelae, and her plasma GFAP concentration during the illness was 0.088 ng/ml.

The first plasma sample available for the analysis was collected 32 h prior to the clinical diagnosis of stroke (fig. 2). This initial GFAP concentration of 1.5 ng/ml was 6.6-fold higher than the normal 95th percentile of pediatric controls (0.227 ng/ml) and 17-fold higher than the sibling control.

Plasma GFAP peaked at 2.83 ng/ml at the time of CT imaging and immediately prior to the first red cell exchange. Blood samples were obtained within 1 h of commencing red cell exchange and within 1 h after the completion of the exchange. Plasma GFAP decreased 42% after the first red cell exchange but decreased by only 13% after the second red cell exchange (fig. 2). The GFAP level in the CSF 1 day after the diagnosis of stroke was 44.6 ng/ml. The most applicable reference value for GFAP in CSF is derived from pediatric acute lymphoblastic leukemia patients <17 years of age in whom CSF GFAP concentrations range from 0.1 to 0.5 ng/ml [10].

Four weeks after the initial stroke and immediately prior to his first scheduled red cell transfusion, plasma GFAP was in the normal range. Subsequently, GFAP levels remained markedly elevated for up to 54 weeks.

Discussion

This patient, with symptoms of gastroenteritis, SCD-related pain, and no clinical evidence of neurologic injury, had a markedly elevated GFAP prior to developing clinical evidence of stroke. This initial elevation was fol-

lowed by a peak GFAP at the time of clinical stroke diagnosis and a decline to normal levels 4 weeks later after 2 red cell exchanges. Elevated GFAP prior to the onset of overt stroke may arise from several causes. There was evidence of prior silent cerebral infarcts on the initial MRI, and elevated GFAP prior to overt stroke may represent elevation from a prior ischemic event. Another possibility is that in the evolution of stroke in SCD, GFAP is an early indicator of brain injury.

The pattern of GFAP levels peaking at the time of stroke and decreasing exponentially immediately after suggests that GFAP tracked the evolution and resolution of stroke. The acute decline in GFAP after red cell exchange may be attributed to treatment efficacy, plasma dilution, a decreased release of GFAP into circulation, or enhanced clearance via renal or proteolytic pathways. Certainly, red cell exchange must dilute plasma GFAP concentrations to some degree because manual red cell exchanges remove whole blood; however, the red cell exchanges reduced GFAP by a disproportionately low amount. The first red cell exchange reduced HbS by 63% and GFAP by 42%, and the second exchange reduced HbS by 46% and GFAP by 13%. Thus, the change in GFAP cannot be entirely explained by plasma dilution. It is likely that an ongoing release of GFAP into the circulation mitigates the dilutive effect of red cell exchange. The elevation of plasma GFAP after effective transfusion and before surgery is more difficult to interpret but is highly suggestive of continuing or recurrent subclinical brain injury.

Despite the reduction in GFAP after the stroke in this child, GFAP became elevated again and remained elevated for a year after his stroke. Chronic elevation of a brain-specific protein may reflect chronic ischemic injury, even though this child had stable disease on serial brain MRIs. Indeed, the presence of moyamoya implies chronic ischemia, even if the regions of infarct on MRI are stable. Thus, elevated GFAP may be a more sensitive marker of subtle brain injury than MRI alone under select circumstances.

GFAP expression is highly restricted to the brain [11], and several studies have taken advantage of this fact to use GFAP as a biomarker of brain injury in other high risk settings, including traumatic brain injury [12], hypoxic ischemic encephalopathy [13], sepsis [14], and brain tumor diagnosis [15]. What is clear from these studies is that GFAP is highly specific for diagnosing brain injury and, therefore, a good measure for ruling in brain injury. Thus, the observation of elevated levels of GFAP that correlate with clinical findings of overt stroke and brain im-

Fig. 2. **a** Serial plasma GFAP levels in a 12-year-old patient with HbSS and acute stroke. A red cell exchange transfusion was performed 5 h after clinical diagnosis and again 47 h later for new neurologic deficits. The dotted line marks the 95th percentile value among 60 healthy pediatric controls. Arrowheads mark the presentation of relevant signs and symptoms. **b** Fifty-four weeks of follow-up GFAP measurements.

aging in this patient make it likely that the GFAP levels reflect brain injury.

The elevation of plasma GFAP in this HbSS patient before the diagnosis of overt stroke raises the possibility that GFAP can detect subclinical brain injury and could

be used as a screening test in ill patients with SCD as approximately 19% of strokes in pediatric SCD patients are associated with antecedent conditions [16]. Transcranial Doppler has been an effective tool for identifying children at risk of stroke, and initiating transfusion therapy in these children leads to a profound reduction in stroke risk [17]. Studies to test whether a blood biomarker can predict stroke risk would be a useful adjunct to current screening because blood is easy to obtain frequently, especially during times of increased risk for stroke. Patients identified at a higher risk could be triaged to brain MRI evaluation or close clinical follow-up.

Brain injury biomarkers could also be used potentially to track treatment efficacy. There is evidence in this report that exchange transfusion is associated with a de-

crease in GFAP. There are too few GFAP measurements after pial synangiosis to make reasonable conclusions about how well GFAP may track the benefits of this procedure, although the GFAP decreased after the surgery. Future studies to correlate posttreatment GFAP levels with neurologic and neurovascular outcomes could yield important prognostic information. Evaluation of GFAP and other potential biomarkers of brain injury in SCD are worthy of further study.

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Note Added in Proof

An additional plasma GFAP level 21 months after stroke remained elevated at 0.535 ng/ml.