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Endothelial and Glycocalyx Proteins as Biomarkers in Delayed Cerebral Ischemia Following Subarachnoid Hemorrhage

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Commentary

Delayed cerebral ischemia (DCI) following aneurysmal subarachnoid hemorrhage (aSAH) remains a relevant issue in the critical care management of patients following aneurysm repair. It is thought to contribute to poor neurologic outcome in up to 30% of patients following SAH [1] and vigilance for delayed neurologic deterioration in the ICU remains high to prevent prolonged periods of unattended ischemic injury. Historically, the pathogenesis of DCI has been attributed primarily to angiographic vasospasm of the arteries of the circle of Willis [1]. The treatment of DCI indeed focuses on mitigating vasospastic events; namely, prophylactic nimodipine, intra-arterial milrinone, balloon angioplasty, blood pressure augmentation and neurointensive care.

While vasospasm unquestionably contributes to delayed neurologic injury and delayed ischemic injury following SAH, other mechanisms of neuronal injury have come to light in recent years, due in large part to preclinical models of the disease and post-mortem examination. As well, attenuation of vasospasm does not reverse or prevent delayed neurologic deterioration, opening the possibility that other events play important roles in delayed neuronal injury after SAH. Of these mechanisms, microthrombosis of the cerebral microvasculature and delayed neuroinflammation are thought to be important contributors [2]. Microthrombi are present in autopsy sections from SAH patients, and high levels of neuroinflammatory proteins are detectable in the CSF of patients with DCI [3]. However, it is not well understood why platelets aggregate so readily following SAH, or why the microthrombi appear so remote from the initial hemorrhage. Similarly, remote areas of brain parenchyma exhibit signs of inflammation of unclear etiology.

In our recently published paper in Neurocritical Care, we sought to gain more information into the mechanisms involved in DCI [4]. Specifically, we focused on the role of the endothelial glycocalyx, a thin layer of glycoproteins lining the apical endothelial surface that shields endothelial-bound receptors from platelet and leukocyte surface proteins [5]. Breakdown of the glycocalyx occurs in a number of pathophysiologic conditions, particularly septic and hemorrhagic shock [6].

Glycocalyx breakdown plays a critical role in leukocyte extravasation across capillary membranes and platelet aggregation in sepsis, and markers of glycocalyx injury are detectable as soluble biomarkers in septic patients [6]. Similarly, we recently published another investigation demonstrating the detection of soluble glycocalyx components in the plasma in an acute head injured population [7]. Thus, we hypothesized that similar glycocalyx injury might occur in DCI, potentially triggering neuroinflammation and microthrombosis.

In sepsis and hemorrhagic shock, glycocalyx breakdown is thought to occur secondary to hypoxia to the endothelium itself, shear stress, or inflammation on the apical side of the endothelium [8,9]. However, a number of glycocalyx components are also targets of proteinases, and their degradation occurs under exposure to these molecules, particularly matrix metalloproteinases (MMPs) [10]. MMPs are generated in abundance following SAH [11] and might mediate glycocalyx injury in these patients.
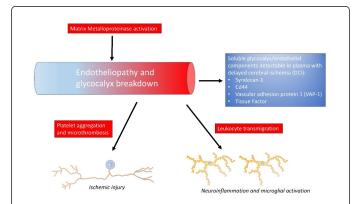
We reported a case series of 3 critical care patients with DCI following SAH. DCI was identified using clinical criteria and scales demonstrating new neurologic deficits, and confirmed by mean transit time mapping on CT perfusion imaging. All patients had parietal lobe ischemia and long-term neurologic deficit at 3 month follow-up. They received standard of care for DCI onset, with one patient receiving intra-arterial milrinone.

Our case series demonstrated that indeed DCI onset was associated with a marked upregulation of matrix metalloproteinases. We detected these increases in both citrated plasma, as well as cerebrospinal fluid (CSF) sampled from in situ external ventricular drains. The largest upregulation was seen in MMP-1, a metalloproteinase which cleaves syndecan-1, the major constituent of the glycocalyx. The study looked as well at soluble markers of endothelial injury and glycocalyx breakdown. A number of biomarkers suggestive of injury to the endothelium were upregulated with DCI onset, including vascular adhesion protein 1 (VAP-1), tissue factor (TF), syndecan-1 (SDC-1) and cd44, the hyaluronic acid receptor also embedded within the glycocalyx layer.

In CSF, we also detected upregulation of soluble vascular cell adhesion molecule (sVCAM), and two markers of neuroinflammation, interleukin-6 (IL-6) and high mobility group box 1 (HMGB1), the latter an emerging damage associated molecular protein (DAMP) signaling the presence of an inflammatory milieu. We confirmed neuronal and glial cell injury with a panel of neuroinjury biomarkers. DCI specifically resulted in upregulation of brain derived neurotrophic factor (BDNF), neurogranin (a marker of dendritic spine injury), and neuron specific enolase (NSE). Taken together, we suggested that MMP-mediated breakdown of the endothelium and glycocalyx layer might mediate some of the processes involved delayed cerebral ischemia (Figure 1).

Some therapies have demonstrated protection of the endothelial glycocalyx, though most of this literature comes from sepsis research. Notable therapies for glycocalyx protection include albumin,

hydrocortisone, heparanase inhibitors, and judicial fluid management (i.e., avoidance of hypervolemia).

Figure 1: Biomarkers of endotheliopathy and glycocalyx injury are detected during delayed ischemia following subarachnoid hemorrhage. Matrix metalloproteinases, upregulated in this case series in both plasma and CSF, might lead to glycocalyx breakdown. Soluble biomarkers of glycocalyx injury and endotheliopathy become detectable as soluble fragments in plasma and CSF. Platelet aggregation as a result of increased platelet-endothelial cell binding and leukocyte transmigration by similar mechanisms leads to microthrombosis and neuroinflammatory responses, respectively.

Ex vivo experiments demonstrate that albumin limits fluid extravasation in the heart, independent of effects on plasma colloidal pressure. Rather, this is mediated by a direct interaction between albumin and glycocalyx components [12]. Even the addition of very low albumin concentrations have been shown to protect endothelial barrier functions. In models of isolated hearts exposed to ischemia/reperfusion injury, hydrocortisone reduced shedding of syndecan-1, heparan sulfate and hyaluronan [13]. Hypervolemia, which is often instituted as part of triple H therapy for vasospasm, actually worsens glycocalyx shedding and may contribute to the findings we observed [14].

Thus, careful fluid management is critical in maintaining endothelial barrier integrity. Lastly, N-desulfated re-N-acetylated heparin (NAH) is a competitive heparanase inhibitor, which prevented endotoxemia-associated glycocalyx injury and subsequent neutrophil adhesion in a model of sepsis [15]. Thus, each of these options might offer glycocalyx protection, though validation of these therapies in SAH experimental models is needed first.

Biomarkers are used in a number of ways that contribute to our understanding of disease: for example, they can serve as diagnostic or prognostic indicators of disease processes, allowing the clinician to detect ongoing tissue injury and act accordingly. The prototypical example is troponin in myocardial ischemia leading to prompt endovascular intervention or surgical bypass.

Another important role of biomarkers is the validation that conjectured mechanisms of disease, sometimes identified by preclinical studies, are present and relevant in humans. The first example can require large data sets to analyze the value of the biomarker and when combined and compared with clinically available information, receiver operating curves are generated to quantify their utility. The latter example, in contrast, requires only small numbers and illustrates the principle that the mechanism is occurring through the demonstration that relevant metabolites are uniquely present. In this study, we suggested that glycocalyx breakdown might be a novel mechanism of delayed neurologic deterioration, allowing leukocyte transmigration into remote brain parenchyma and platelet aggregation, leading to microthrombi formation.

We illustrated in a small number of patients that there was evidence that supported this suggestion by measuring the presence of relevant biomarkers. Identification of these type of mechanistic biomarkers in patients who have active ischemic injury is among the most critical steps in understanding the pathogenesis of DCI, augmenting pre-clinical animal work and cell culture models probing specific cell signaling pathways. The next stage would be to measure and calibrate the impact of this pathophysiologic process of endothelopathy and glycocalyx injury in mediating DCI and the attendant neurologic deficit; ideally this would be followed by trials of targeted therapeutic strategies.

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