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Why have we not yet developed a simple blood test for TBI?

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In recent years, traumatic brain injury (TBI) has emerged as a rapidly growing public health challenge. Annually, approximately 1.7 million people will sustain a TBI in the USA and WHO has named TBI the leading cause of death and disability in young adults worldwide, predicting it will become the third leading cause of death in the general population by 2020. The medical community currently relies on clinical examination and various neuroimaging modalities for the diagnosis of TBI; however, these methodologies are often confounded by altered patient mental status and are particularly poor at identifying mild-to-moderate injury. Despite decades of basic and clinical research, and the identification of hundreds of biochemical markers, presently there is no blood test to objectively assess TBI severity. Recent work suggests treatment-induced variance in the brain's glymphatic clearance may be responsible for the breakdown between biomarker discovery and clinical translation.

Traumatic brain injury (TBI) is a disease entity with inherent diagnostic and prognostic challenges. This fact can be attributed to the non-specific symptomatology and variable presentation accompanying TBI. The clinical picture of TBI can include unique combinations of headache, nausea, dizziness, loss of consciousness, anxiety, memory impairment, attention deficits and even sleep cycle disorders [1–3]. The burden of the 1.7 million annual TBIs in the USA falls disproportionately on a young population due to athletic participation and military service [4]. Recently, an association between patients who have sustained repetitive head impacts or blast injuries and the development of chronic neurodegeneration has been hypothesized based on a growing body of evidence [5]. As a consequence, there is an increasing urgency to develop new diagnostic modalities that will allow for the accurate identification of at-risk patients and the initiation of preventative therapy early in the disease course.

The past three decades have seen considerable effort dedicated to the discovery of objective injury biomarkers that accurately reflect the presence, as well as extent, of brain injury. Clinical examination, specifically, Glasgow Coma Scale scoring, is the most frequently employed post-traumatic assessment tool, however, its use is frequently confounded by age, polytrauma and altered patient mental status due to intoxication or the necessity for medical sedation and intubation [2,3,6]. Neuroimaging modalities, including CT and MRI, are also routinely utilized in the acute phase following injury. These techniques, while effective in identifying head injury resulting in hemorrhage or mass effect, inaccurately diagnose and prognose TBIs with diffuse injury in the absence of elevated intracranial pressure [5,7,8]. Together, clinical assessment and neuroimaging lack the sensitivity to reliably diagnose mild TBI, which accounts for 75–90% of all neurotrauma cases [9–11]. In more recent years, research efforts have been recalibrated in the pursuit of biochemical markers of brain injury. The cerebrospinal fluid (CSF), due to its close proximity and interaction with CNS structures, has been regarded as the ideal biofluid for the assay of molecules associated with TBI. CSF-based assays have improved sensitivity to early brain injury and enhanced specificity for

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TBI overall [2]. The invasive nature of lumbar puncture and ventriculostomy, however, represents a considerable drawback of this approach and has catalyzed the development of a blood test for injury marker detection [1].

The advantages of a serum-based assay include the low cost of administration, broad availability, non-invasive nature and the potential for repeat sampling to follow disease progression and response to therapy [1]. S100β (10.5 kDa), perhaps the most extensively studied marker of TBI, is a member of the S-100 family of low molecular weight Ca^{2+}-binding proteins [3,7,9]. S100β has been demonstrated to be an exquisitely sensitive indicator of blood–brain barrier (BBB) dysfunction [12–14], and with a negative predictive value for CT-positive intracranial lesions approaching 100% it is most useful in ruling out the presence of brain trauma [15,16]. Constitutively expressed in the cytoplasm of astrocytes, S100β is also found in Schwann cells, as well as non-nervous tissues including chondrocytes, adipocytes and melanoma cells [7]. Consequently, this molecule has been shown to be elevated in the serum of patients with extracranial trauma in the absence of head injury [17]. Additionally, there have been many studies demonstrating that BBB opening, due to hyperosmotic disruption or endarterectomy, results in an increase in serum S100β levels independent of primary brain pathology [12,13,18]. Most recently, Papa *et al.* published a prospective study following a large cohort of general trauma patients with and without mild-to-moderate TBI. Here, it was found that S100β possessed specificity for brain trauma of only 5% and a positive predictive value for CT-identifiable brain lesions of only 11% [15].

This deficit of specificity has inspired a search for molecular entities that better reflect the pathobiology of TBI. Glial fibrillary acidic protein (GFAP, 52 kDa) is the primary intermediate filament within the astrocytic cytoskeleton [7]. Owing to its exclusive expression in astrocytes, the previously discussed study from Papa *et al.* found that GFAP possesses specificity for brain injury of 55%, demonstrating superior performance over S100β as a marker of TBI [15]. Disappointingly, this same study found that when a serum screen for GFAP was positive, CT-evident brain lesions were only found in 20% of patients [15]. Consequently, the gains afforded by improved marker selection only marginally enhance the diagnostic utility of blood-based assays and have left many researchers offering alternative hypotheses for the diagnostic failure of these molecules.

Several possible explanations have been proposed for this breakdown between discovery and translation including that brain proteins levels, such as that of tau or cleaved tau, may be so low in the blood that insufficient assay sensitivity has allowed these molecules to elude detection [1]. Additionally, it has been demonstrated that in the peripheral blood these endogenous brain substances can undergo proteolytic degradation, hepatic and renal clearance and can be bound to carrier proteins resulting in serum levels that may not accurately portray the presence or extent of brain trauma [1].

Alternatively, an understanding of how these molecules move from the brain where they are released and into the blood where they are detected may reconcile the disconnect between marker identification and diagnostic performance. It has been demonstrated that following BBB opening, either due to hyperosmotic disruption, endarterectomy, cerebral microvascular disease, tumor metastasis or TBI, serum levels of brain-endogenous proteins, such as S100β, rapidly rise [12–14,18,19]. An unanswered question, however, is how neuronal and glial proteins released into the extracellular space local to the TBI are able to migrate into the more distant perivascular channels prior to crossing the brain endothelium. The movement of solutes within the interstitial space of the brain is the consequence of two different processes: diffusion and bulk flow (transport resulting from hydrodynamic fluid flow) [20]. In a seminal study by Sykova and Nicholson, it was found that it would take 10 h for an albumin-sized molecule to diffuse 1 mm within the extracellular space of the brain [20]. Furthermore, Cserr *et al.* discovered that different molecular weight polyethylene glycols and albumin were cleared from the brain at equal rates [21]. Taken together, the conclusion of these studies, and the consensus within the literature, is that molecular movements within the brain's interstitium are not diffusion limited, but instead governed by bulk flow.

Work from our group characterizing the recently discovered glymphatic pathway provides evidence that this convective bulk flow process drives interstitial fluid from the brain parenchyma into perivenous spaces [22]. These perivascular channels then act similar to a distribution center for brain-derived solute, allowing at least a portion of these molecules to pass across the BBB due to receptor-mediated transport [23] or pathological opening [24], while the remainder are driven by bulk flow back into the subarachnoid CSF compartment [22]. Subarachnoid CSF is then drained from the cranium along the myelin sheaths of cranial and spinal nerve roots, ultimately being absorbed by perineural lymphatic vessels [25,26]. In studies in lower mammals including cats, rabbits and rodents, it has been estimated that up to 30% of CSF drains through the cribriform plate via the olfactory bulbs and olfactory mucosa to be picked up by the deep cervical lymph [26]. In humans, the proportion of brain solute cleared across the BBB versus any of the alternative egress site remains poorly defined, however, it is clear that glymphatic-driven bulk flow is important for the delivery of these molecules to the various points of efflux.

In a recent publication, we showed that interventions that suppress interstitial fluid flow through the glymphatic pathway would, as a consequence, prevent the appearance of injury biomarkers in the blood. Here, we found that aquaporin-4 deletion, inhibition of CSF production with acetazolamide therapy, elimination of intracranial pressure by opening the skull and sleep deprivation were each capable of reducing blood biomarker concentrations to trauma-naïve levels [27]. Thus, we concluded that clinically relevant manipulation of glymphatic clearance capacity, using four mechanistically independent approaches, would make the accurate interpretation of serum biomarker levels impossible. It is then conceivable that genetic, environmental and iatrogenic influences on the kinetics of

glymphatic clearance will similarly act to prevent the accurate clinical interpretation of the level of any injury marker, regardless of its identity.

We have not yet developed a blood test that is capable of accurately determining the presence and grading the severity of TBI because at present we do not have a clinical tool for measuring glymphatic-derived convective bulk flow in humans. The development of a metric of glymphatic pathway function, either utilizing neuroimaging or the assay of biofluids, will provide a normalization index for more traditional serum-based biomarker assessments. Establishing a panel of biomarkers combining measurement of glymphatic clearance with biochemical assays and clinical assessment of factors including loss of consciousness and amnesia likely offers the best hope for improving the diagnostic performance of the multitude of injury markers presently under investigation [3]. When this goal is achieved, it will be possible to begin to evaluate the role of each of these molecular entities in the pathophysiology of TBI, and further it may even be possible to determine the contribution of glymphatic failure in the evolution of this disease process [28].

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References

1. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol 2013;9(4):201-10
2. Sharma R, Laskowitz DT. Biomarkers in traumatic brain injury. Curr Neurol Neurosci Rep 2012;12(5):560-9
3. Jeter CB, Hergenroeder GW, Hylin MJ, et al. Biomarkers for the diagnosis and prognosis of mild traumatic brain injury/concussion. J Neurotrauma 2013;30(8):657-70
4. Faul M, Wald MM, Coronado VG. Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002–2006. (Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, Atlanta (GA), 2010)
5. DeKosky ST, Blennow K, Ikonomovic MD, et al. Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. Nat Rev Neurol 2013;9(4):192-200
6. Di Battista AP, Rhind SG, Baker AJ. Application of blood-based biomarkers in human mild traumatic brain injury. Front Neurol 2013;4:44
7. Kövesdi E, Lückl J, Bukovics P, et al. Update on protein biomarkers in traumatic brain injury with emphasis on clinical use in adults and pediatrics. Acta Neurochir (Wien) 2009;152(1):1-17
8. Mondello S, Muller U, Jeromin A, et al. Blood-based diagnostics of traumatic brain injuries. Expert Rev Mol Diagn 2011;11(1):65-78
9. Papa L, Ramia MM, Kelly JM, et al. Systematic review of clinical research on biomarkers for pediatric traumatic brain injury. J Neurotrauma 2013;30(5):324-38
10. Shen S, Loo RR, Wanner IB, et al. Addressing the needs of traumatic brain injury with clinical proteomics. Clin Proteomics 2014;11(1):11
11. Eierud C, Craddock RC, Fletcher S, et al. Neuroimaging after mild traumatic brain injury: review and meta-analysis. Neuroimage Clin 2014;4:283-94
12. Marchi N, Angelov L, Masaryk T, et al. Seizure-promoting effect of blood-brain barrier disruption. Epilepsia 2007;48(4):732-42
13. Kanner AA, Marchi N, Fazio V, et al. Serum S100beta: a noninvasive marker of blood-brain barrier function and brain lesions. Cancer 2003;97(11):2806-13
14. Blyth BJ, Farahvar A, He H, et al. Elevated serum ubiquitin carboxy-terminal hydrolase L1 is associated with abnormal blood-brain barrier function after traumatic brain injury. J Neurotrauma 2011;28(12):2453-62
15. Papa L, Silvestri S, Brophy GM, et al. GFAP out-performs S100beta in detecting traumatic intracranial lesions on computed tomography in trauma patients with mild traumatic brain injury and those with extracranial lesions. J Neurotrauma 2014;31(22):1815-22
16. Unden J, Romner B. Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: an evidence-based review and meta-analysis. J Head Trauma Rehabil 2010;25(4):228-40
17. Anderson RE, Hansson LO, Nilsson O, et al. High serum S100B levels for trauma patients without head injuries. Neurosurgery 2001;48(6):1255-8
18. Mussack T, Hauser C, Klauss V, et al. Serum S-100B protein levels during and after successful carotid artery stenting or carotid endarterectomy. J Endovasc Ther 2006;13(1):39-46
19. Vogelbaum MA, Masaryk T, Mazzone P, et al. S100beta as a predictor of brain metastases: brain versus cerebrovascular damage. Cancer 2005;104(4):817-24
20. Sykova E, Nicholson C. Diffusion in brain extracellular space. Physiol Rev 2008;88(4):1277-340
21. Cserr HF, Cooper DN, Suri PK, et al. Efflux of radiolabeled polyethylene glycols and albumin from rat brain. Am J Physiol 1981;240(4):F319-28
22. Iliff JJ, Wang M, Liao Y, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. Sci Transl Med 2012;4(147):147ra111
23. Bell RD, Sagare AP, Friedman AE, et al. Transport pathways for clearance of human Alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system. J Cereb Blood Flow Metab 2007;27(5):909-18

24. Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood-brain barrier pathophysiology in traumatic brain injury. Transl Stroke Res 2011;2(4):492-516

25. Johnston M, Papaiconomou C. Cerebrospinal fluid transport: a lymphatic perspective. News Physiol Sci 2002;17: 227-30

26. Bradbury MW, Cole DF. The role of the lymphatic system in drainage of cerebrospinal fluid and aqueous humour. J Physiol 1980;299:353-65

27. Plog BA, Dashnaw ML, Hitomi E, et al. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J Neurosci 2015;35(2): 518-26

28. Iliff JJ, Chen MJ, Plog BA, et al. Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. J Neurosci 2014;34(49): 16180-93