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Finding effective biomarkers for pediatric traumatic brain injury

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Abstract:

As traumatic brain injury (TBI) continues to affect children and young adults worldwide, research on reliable biomarkers grows as a possible aid in determining the severity of injury. However, many studies have revealed that diverse biomarkers such as S100B and myelin basic protein (MBP) have many limitations, such as their elevated normative concentrations in young children. Therefore, the results of these studies have yet to be translated to clinical applications. However, despite the setbacks of research into S100B and MBP, investigators continue to research viable biomarkers, notably glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase L1 (UCH-L1), as possible aids in medical decision making. Studies have revealed that GFAP and UCH-L1 actually are better predictors of injury progression than the before-mentioned biomarkers S100B and MBP. In addition, UCH-L1 has demonstrated an ability to detect injury while CT is negative, suggesting an ability to detect acute intracranial lesions. Here, we evaluate research testing levels of GFAP and UCH-L1 on children diagnosed with TBI and compare our results to those of other tested biomarkers. In a recent study done by Hayes *et al.*, GFAP and UCH-L1 demonstrated the potential to recognize children with the possibility of poor outcome, allowing for more specialized treatments with clinical and laboratory applications. Although studies on GFAP and UCH-L1 have for the most part warranted positive results, further studies will be needed to confirm their role as reliable markers for pediatric TBI.

Key words:

Biomarkers, brain injury, serum, traumatic brain injury

Ubiquitin C-terminal Hydrolase-L1 and Glial Fibrillary Acidic Protein as Potential Biomarkers for Pediatric Traumatic Brain Injury

Traumatic brain injury (TBI), a prominent cause of acquired disability and mortality, affects many children and young adults across the globe.[1] Following TBI, a child can experience two outcomes: on the one hand, the child can display superior recovery rates to adults, on the other hand, they can demonstrate extended and exacerbated symptoms than those experienced by older patients.[2,3] While knowledge of potential mortality following TBI and improved awareness of the biological mechanisms that contribute to the heightened vulnerability of the pediatric brain have increased in the past decade,[3,4] pediatric TBI continues to test clinicians. Therefore, reliable biomarkers indicative of damage to the central nervous system (CNS) in combination with other currently available clinical data would greatly aid medical decision-making.[5]

Recently, a great number of investigations have attempted to find viable biomarkers, focusing on blood-based structurally and pathobiologically diverse biomarkers like S100B and myelin basic protein (MBP). Many of these studies revealed noticeable differences in biomarker concentration between affected and healthy children, the affected displaying significantly elevated concentrations. However, these results have yet to be translated to clinical applications,[6-8] possibly due to the many limitations of biomarkers, such as elevated normative concentrations in young children and a belated presence in serum following the injury.[9-11] Therefore, many investigators are attempting to discover novel blood-based biomarkers that overcome the previously discussed limitations.

Ubiquitin C-terminal hydrolase (UCH-L1), a proteolytically stable and plentiful protein present almost solely in neuronal cytoplasm, has been found to increase in concentration in serum after TBI.[12-14] UCH-L1 demonstrates increased serum concentration in correlation with outcome, as demonstrated by a previous exploratory study.[15] Another more well-known protein, glial

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fibrillary acidic protein (GFAP), is a long-standing marker of glial impairment in multiple neurologic diseases.[16] Several studies on adults[14,17,18] and children[19,20] have demonstrated increased serum GFAP in the blood after TBI. These discoveries suggest the potential of UCH-L1 and GFAP to identify cerebral injury and aid in clinical decisions using the biomarkers as indications of TBI severity.

Ubiquitin C-terminal Hydrolase-L1 and Glial Fibrillary Acidic Protein Capable of Detecting Acute Intracranial Lesions

A recent study conducted by Mondello *et al*. compared serum concentrations of UCH-L1 and GFAP between children who suffered mild to severe TBI and unharmed controls to determine if the levels of the two markers were significantly elevated in injured children.[21] In addition, this study compared their performance to two extensively investigated biomarkers, S100B and MBP, using previously published data.[22-24]

To properly assess and treat pediatric TBI, objective biomarkers are needed to predict and track the progression of TBI. With this in mind, serum concentrations of UCH-L1 and GFAP were evaluated to determine if they could be possible candidates. Testing involved 45 children clinically diagnosed with TBI (Glasgow Coma Scale 3–15) along with 40 healthy children. Levels of GFAP and UCH-L1 in comparison with S100B and MBP, two additional blood biomarkers, were examined. First noting the difference in concentration of GFAP and UCH-L1 between the controls and the children diagnosed with TBI, the researchers also showed a direct relationship between biomarker levels and the severity of the brain injury. In addition, they found that although UCH-L1 is the only neuronal biomarker with the ability to identify acute intracranial damage, elevated levels of both markers in TBI patients with normal computed tomography (CT) scans revealed their ability to demonstrate the presence of microstructural injuries not detected on a CT scan. Furthermore, in comparison to the levels of S100B and MBP, concentrations of GFAP and UCH-L1 acted as more accurate indicators of poor outcomes for patients. Although further studies are needed, these results suggest GFAP and UCH-L1 should be used as biomarkers for pediatric TBI.

Validation of Biomarker-Based Therapy

Interestingly, Hayes *et al.* discovered not only that TBI subjects with intracranial injury had the highest concentrations of GFAP and UCH-L1, but also that patient with a skull fracture or negative CT displayed increasing concentrations as well. This intriguing observation in the study proposes the possibility of undetected brain damage. Moreover, an increasing number of studies have revealed that, compared to magnetic resonance imaging (MRI), CT is a poor method to identify, quantify, and distinguish acute lesions and pathophysiological alterations that occur as a result of TBI.[23,25,26] In addition, Hayes along with other investigators have provided evidence of increased serum biomarker concentrations in CT-negative TBI patients, further putting into question the viability of CT for patients with persisting symptoms or subtle abnormalities.[27-30] Therefore, the detected biomarker release in the cases involving skull fracture or a negative CT may have risen from molecular perturbation, limited structural damage, or specific pathoanatomic types of TBI, such as diffuse axonal injury or microbleeds, that CT did not previously detect.[31-33] Due to GFAP and UCH-L1's capabilities to detect microbleeds and acute lesions, they may prove effective in diagnostic imagining in pediatric TBI. In addition, these findings put into question the reliability of CT as a dependable method to detect the incidence of brain injury and judge the success of biomarkers. However, these observations by no means provide sufficient conclusions as more studies are required to validate these markers in combination with MRI and other innovative imaging.[21]

These observations suggest that these biomarkers may prove effective in risk determination, supporting their use to classify injury severity perhaps in combination with clinical and imaging data. These findings propose a classification system that may prove to be highly beneficial for pediatric use in TBI using acute serum markers. This may not only be valuable in diagnosis and prognosis but also could reveal information on the injury-specific and patient-specific vulnerability as it relates to translation to clinical trials.[34]

The development of new and available technologies with the necessary precision and sensitivity will have the capabilities to fully demonstrate the dispersal of biomarkers in the bodies of healthy people, as well as the capability to identify minute changes in biomarker concentrations in individuals suffering from TBI.[35,36] Specifically in Mondello *et al*.' study, they were able to identify low biomarker levels utilizing GFAP and UCH-L1 assays.[21] In these patients, a strong and direct relationship was detected between age and serum UCH-L1. While it has been observed that UCH-L1 levels increase with age in healthy adults, it remains to be determined if the same relationship exists in children and young adults.[37] The observation in Mondello *et al*.' investigation[21] that the serum UCH-L1 changed with age in children can be best explained by the fact that infant brains are underdeveloped and the blood–brain barrier has a higher permeability.[38] It can also be explained by noting the distinctive age-related changes in cerebral biology and continuing CNS advancement associated with early stages of life. While it has been demonstrated that UCH-L1 plays a role in neuron survival and function, other recent investigations have shown that it plays a role in guiding neural progenitor cells through neurogenesis and differentiation.[39]

It is worth noting that while GFAP and S100B can serve as glial markers, the results of Mondello *et al*.' demonstrate that GFAP is a more effective biomarker for TBI.[21] The best explanation for S100B's limitations are its dependence on age, especially in young children, and its lack of particularity with extracranial damage.[40-43] Further investigations on children with acute orthopedic damage separate from the CNS would be essential in helping determine the specificity of UCH-L1 and GFAP in diagnosing pediatric TBI.

In summary, GFAP and UCH-L1 present as viable candidates for biomarkers for pediatric TBI. Considering the traumatic effects of TBI on the developing brain, such as incomplete neural connectivity, brain maturation, and impaired functional

capabilities, serum biomarkers' capabilities to improve diagnostic precision and serve as evidence of TBI in the case of a normal CT scan make them important candidates for further research.[44] In addition, serum biomarkers could also serve as potential guides for selecting patients for advanced neuroimaging. While both are indicators of TBI, only UCH-L1 can function as a biomarker for acute intracranial lesions. Moreover, UCH-L1 and GFAP have the potential to recognize children with the possibility of poor outcome, thereby granting further opportunities for more specialized treatments with clinical and laboratory applications. Further studies will need to be conducted to confirm the role of GFAP and UCH-L1 and their utility as markers of pediatric TBI.

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Conflicts of interest
Ronald L Hayes owns stock, receives compensation from, and is an executive officer of Banyan Biomarkers, Inc., and as such, may benefit financially as a result of the outcomes of this research or work reported in this publication.

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