Detection of neuroinflammation in the urine of patients with severe traumatic brain injury

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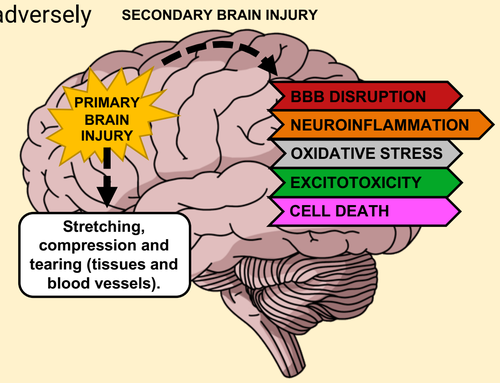
Introduction

Over 50% of people with severe traumatic brain injury (TBI) will experience further decline in their daily lives or die within 5 years of their injury.[1]

Preclinical and clinical TBI outcomes are adversely influenced by neuroinflammation.[2,3]

Cellular neuroinflammatory components, microglia and macrophages, mediate the release of various proteins, which can be measured in biofluids as a reflection of underlying neuropathology. [3]

TBI biomarker research has largely focused on invasive biofluids, cerebrospinal fluid and peripheral blood, with limited progress.[4]



No study has looked for neuroinflammatory markers in the urine of severe TBI patients

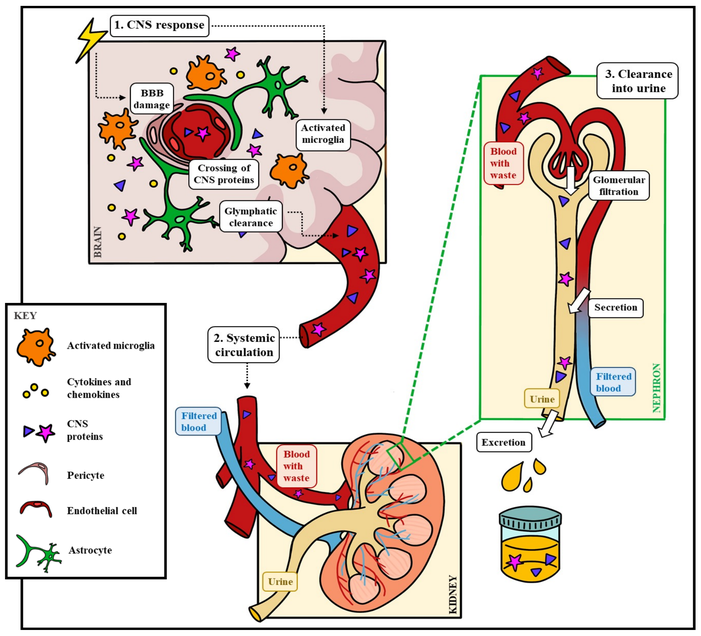


Figure 2. Proposed passage of central nervous system (CNS) proteins from the brain to the urine.

Following TBI, CNS proteins can enter the systemic circulation across a damaged blood-brain barrier (BBB) or via the glymphatic system.

Accumulated proteins in the systemic circulation are cleared into the urine through glomerular filtration or secretion.

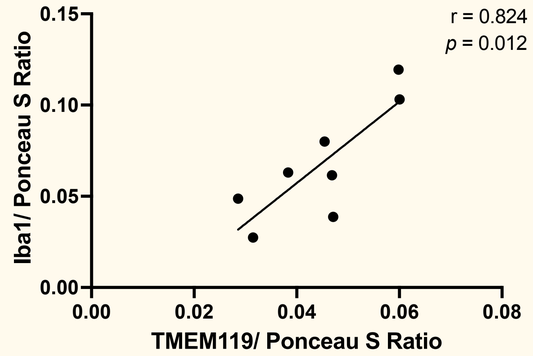
Results

Detection of urinary TMEM119 and Iba1 in severe TBI patients for 7 days post injury

	Samples detected (%)
TMEM119	65%
Iba1	100%

TMEM119 and Iba1 were detected in urine samples, indicating the presence of the markers, components of microglia and macrophages, in the urine.

Correlation between Iba1 and TMEM119 in all samples across day 0 to day 7.

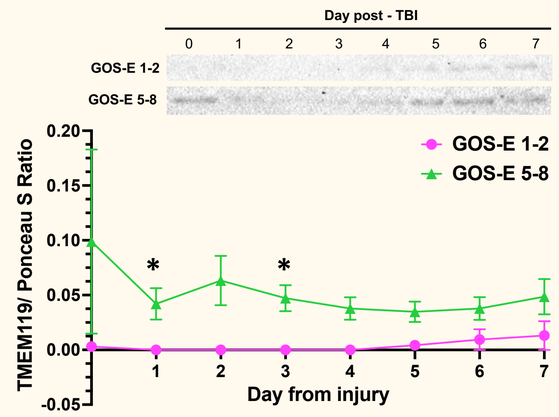


Significant positive correlation between total urinary Iba1 and TMEM119 expression across day 0 to day 7.

Although Iba1 is a non-specific marker, a positive relationship with TMEM119, a microglia-specific marker, further supports the presence of microglia/ macrophage proteins.

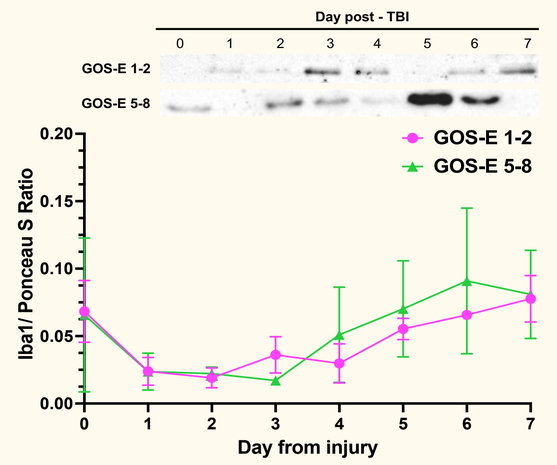
Temporal expression of TMEM119 and Iba1 in the urine of severe TBI patients across 7 days post injury

N = 5 GOS-E 1-2 group (death – vegetative state)
N = 6 GOS-E 5-8 group (mild – moderate disability)



TMEM119 expression was undetectable for patients with poor outcomes until day 6.

Significant differences in urinary TMEM119 expression on day 1 and 3 between the two groups.



Iba1 levels showed a gradual increase in the subacute phase for both groups.

* $p < 0.05$

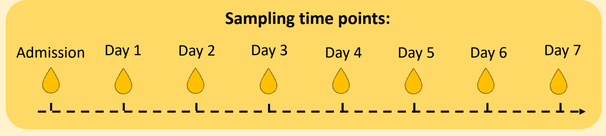
TMEM119 was undetectable until the subacute phase (> day 4) in the GOS-E 1-2 group, suggesting minimal microglial activity and a lag in producing a sufficient neuroinflammatory response. The GOS-E 5-8 group may be benefitting from an acutely heightened neuroinflammatory response.

Iba1 is a non-specific marker of neuroinflammation and peripheral inflammation, which may account for similar expression levels in both GOS-E groups. Increasing levels of Iba1 expression in the subacute phase indicates continued inflammation at later time points following TBI.

Methods

Western blot analysis to quantify urinary levels of microglia and macrophage protein markers as an indication of underlying neuroinflammation:

1. Macrophage/ microglia marker **ionised calcium-binding adaptor molecule (Iba1)**
2. Microglia marker **transmembrane Protein 119 (TMEM119)**



N = 16 severe TBI patients (GCS < 8)

Conclusion

Neuroinflammatory markers can be detected in the urine after severe TBI.

Patients with favourable outcomes (GOS-E 5-8) had significantly higher levels of TMEM119 in urine compared to patients with unfavourable outcomes (GOS-E 1-2) during the acute injury phase (day 1 and day 3).

TBI biomarker research may benefit from screening urine, as a biologically stable, abundant and non-invasive alternative that can be collected at ease.

Future Directions

A panel of several neuroinflammatory markers can provide a clearer indication of the temporal profile of various components of the neuroinflammatory response post TBI.

A larger sample size may further highlight important changes in marker expression levels and its relationship to patient outcomes.

References

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