Single molecule array glial fibrillary acid protein and tau are increased in plasma after traumatic brain injury

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BACKGROUND

- Glial fibrillary acid protein (GFAP) and microtubule-associated protein tau are described as promising diagnostic and prognostic biomarkers in traumatic brain injury (TBI).
- However, current assays are not sensitive enough to detect low levels of GFAP and tau in plasma.
- Single Molecule Array (Simoa) is a novel technology, which employs highly sensitive immunoassays and allows accurate measurements of candidate biomarkers in blood. The digital approach makes use of arrays of femtoliter-sized reaction chambers that can isolate and detect single enzyme molecules and provides a 1000-fold improvement in sensitivity over traditional immunoassays.

OBJECTIVES

- 1) To validate Simoa technology of ultrasensitive assays for biomarkers implicated in mild TBI (mTBI).
- 2) To compare tau and GFAP levels in mild and moderate-to-severe TBI (m-sTBI) with healthy controls.

MATERIALS AND METHODS

- Ultra-sensitive assays to GFAP and total tau were developed, using specific capture and detection antibodies. The limit of detections of the total tau assay in blood is 0.02 pg/ml.
- Plasma tau and GFAP were measured in samples from 18 complicated mTBI (age 41 ± 15 yrs) and 13 m-sTBI (age 31 ± 12 yrs) participants in the Citicoline Brain Injury Trial (COBRIT), collected within 24 hrs after the injury, and compared to those of healthy volunteers (n=28, age 43 ± 9.5 yrs) without history of TBI and normal brain MRI.

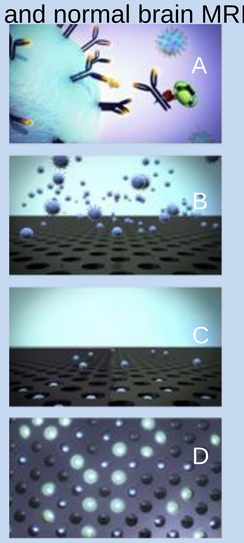

Diagram of loading, sealing, and imaging of single paramagnetic beads in arrays of femtolitre-sized wells: (A) Beads - a fraction of which is associated with captured and enzyme-labeled protein molecules - are introduced into the array. (B) Beads settle by gravity onto the surface of the array. A fraction of the beads fall into microwells while the remainder remain on the surface. (C) Oil is introduced into the channel to displace the aqueous medium and excess beads, and seal the wells. (D) Sealed wells are imaged. Fluorescent signals are generated in sealed wells that contain beads associated with captured and enzyme-labeled protein molecules. www.quanterix.com

RESULTS

Increase of GFAP and tau in mild and moderate-to-severe TBI

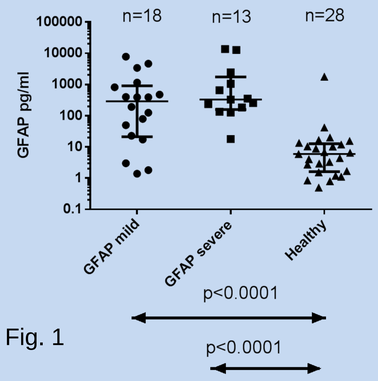

Fig. 1

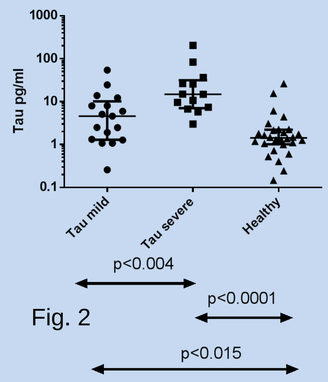

Fig. 2

ROC curve: ROC Analysis GFAP

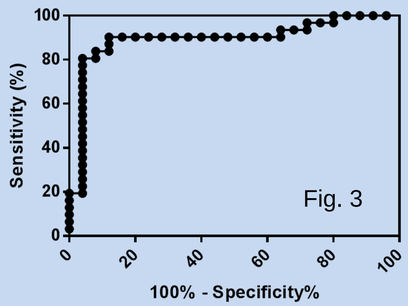

Fig. 3

ROC curve: ROC Analysis Tau

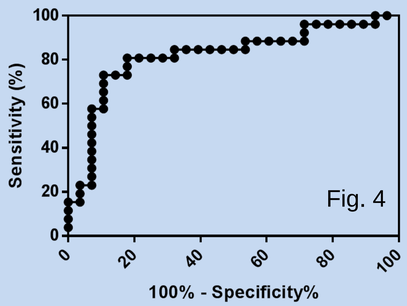

Fig. 4

GFAP and tau in mild TBI

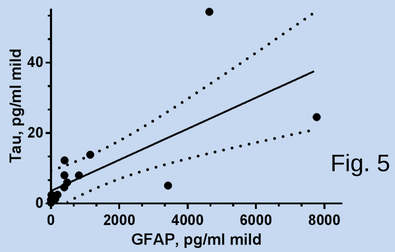

Fig. 5

Spearman r 0.9 (95% CI 0.76-0.97), p<0.0001

GFAP and GOSE 180 days

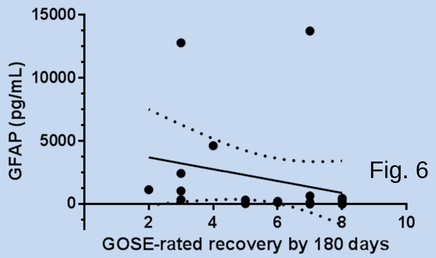

Fig. 6

Spearman r -0.426 (95% CI - 0.73 to 0.02), p<0.054

GFAP was increased in mTBI (mean (IQR) 290 (21-903)) pg/ml and in m-sTBI (mean (IQR) 329 (158-1744)) pg/ml compared to healthy volunteers (mean (IQR) 6 (1.6-12.7)) pg/ml (p<0.0001 for both) (Fig. 1).

Tau was similarly increased in mTBI (mean (IQR) 4.6 (1.3-10)) pg/ml and in m-sTBI (mean (IQR) 14.8 (7-31) pg/ml compared to healthy (mean (IQR) 1.4 (1-2.2)) pg/ml (p<0.015 and p<0.0001, respectively) (Fig. 2).

Tau was increased in m-sTBI compared to mTBI (p<0.004), while no difference in GFAP by TBI severity (p<0.33) was found.

Receiver-operator characteristic (ROC) analysis found that the area under curve (AUC) for tau was 0.82 (95% CI 0.69 - 0.94), when for GFAP it was 0.86 (95% CI 0.80 - 0.99) (Fig. 3 - Fig. 4).

There was a strong positive correlation (r = 0.9 (95% CI 0.76-0.97, p<0.0001)) between tau and GFAP in mTBI (Fig. 5), when no correlations between tau and GFAP in m-sTBI or in healthy controls were found.

There was a trend to moderate negative relationship between GFAP and GOSE 180 days after the injury (r = -0.426 (95% CI -0.73-0.02), p<0.054)) (Fig. 6), when no correlation between tau and GOSE was found.

CONCLUSIONS

Tau and GFAP are increased after mild and moderate-to-severe TBI compared to healthy controls. Increase of tau 24 hrs after the injury discriminates between mild and moderate-to-severe TBI. Pilot data suggest that plasma tau and GFAP measured by Simoa may be useful as biomarkers of TBI in acute phase.

FUTURE WORK

Validate GFAP and total tau Simoa findings on larger sample size including serial samples of mild, severe and of hyperacute TBI patients.

REFERENCES

www.quanterix.com, Rissin et al, 2010, Nature Biotechnology 28 (6), 595-9.

ACKNOWLEDGEMENTS

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