Background

Blood-based biomarker assessment of mild to severe sports-related traumatic brain injury represents a desirable goal as an adjunct for assessing severity of injury and fitness to return to play. Methods for measuring the brain protein Tau in serum and plasma have until recently been unavailable. However, highly sensitive measurement of peripheral total Tau is now available via Single Molecule Array (Simoa™) digital immunoassay. In this pilot study we examined plasma total Tau levels in professional hockey players who had suffered a sports-related concussion. Post-concussion plasma Tau levels were followed over approximately six days and were compared with Tau levels measured in a cohort of hockey players prior to the start of the hockey season.

Methodology

A ultrasensitive ELISA for total tau was developed on an automated analyzer. The limit of detection of the total tau assay in blood is 0.02 pg/ml with overall inter-day precision of less than 10%, which represents a 1000 fold improvement in sensitivity over current assay. The Simoa assay used reagents similar to those for a bead-based ELISA conventional ELISA. Tau-specific capture antibody was immobilized on para-magnetic beads, and detection antibody was biotinylated. Sandwich immunocomplexes were formed and then labeled with streptavidin-conjugated beta-galactosidase. Beads with the labeled immuno-complexes were isolated and sealed in individual microwells of the array containing fluorescent substrate. Well arrays were imaged with a CCD camera. Enzyme-labeled beads that converted substrate into fluorescent product over time were considered to be "on" for purposes of digital counting. The entire range of signal was determined using imaging analysis software to determine the Average Enzyme per Bead (AEB), the unit of measurement for Simoa. A standard curve relating the AEB output to Tau concentration was used to determine the sample concentration. The digital Tau immunoassay was evaluated for ability to measure Tau in pre-season and post-concussion time course plasma samples collected from professional hockey players. Serial blood samples were obtained from 28 hockey players following concussion ranging from mild to severe. Aliquots of plasma from the blood samples were tested by digital immunoassay (LoD 0.03 pg/ml); Plasma Tau levels were measured over 150 hours and compared with plasma Tau levels obtained from pre-season blood draws. Time course profiles and plasma Tau levels were compared with concussive severity.

Postanlysis results compared Simoa data with Return to Play calls ("Return to Play call" refers to the time at which a player who has been assessed for TBI is allowed to resume sport activity), Return to Play call subdivided the TBI samples into three categories: < 6 days (mild), 7-10 days (moderate), and > 10 days (severe).

Results

Plasma Tau was significantly higher in post-concussion samples compared with pre-concussion samples (p<0.0005). Circulating plasma Tau levels were significantly elevated compared with pre-season levels at the observed post-concussion timepoints (see Fig. 2 for p values). Overall mean plasma Tau dropped 30% (significant at p<0.0011), from 18.59 pg/ml to 13.24 pg/ml during the first 12-hour period, with approximately 40% of donors exhibiting a >2-fold drop. 20/25 donors exhibited a Tau drop within 12 hours, while 4/25 donors exhibited an increase. Further declines in plasma Tau were not statistically significant between 12 hours and 6 days (p=0.155).

Plasma Tau levels for up to 6 days were significantly elevated compared with plasma Tau levels from the cohort of pre-season hockey players (p=0.0006). Based on the Simoa results for concentrations of plasma Tau divided into three subcategories of mild, moderate, and severe, the Return to Play calls underestimated the severity of concussion based on plasma Tau levels in 16/28 (57%) of TBI cases.

Fig. 1. Plasma Tau in pre-season vs. post-season concussion. The Y-axis depicts plasma Tau concentration in pg/ml as calculated from a standard curve of Tau 383, using the Tau Simoa assay on the Simoa HD-1™ Analyzer.

Conclusions

These data are the first to examine sports-related post-concussion changes in plasma Tau. The results indicate that plasma Tau is significantly elevated following mild to severe concussion and remains elevated compared to pre-season levels for up to 6 days post-concussion. There is a large discrepancy between Return to Play calls and Simoa-measured Tau levels, which can be correlated to mild, moderate, and severe TBI. This discrepancy suggests that an analytical method using Tau as a biomarker may help clarify or contribute to better Return to Play decisions. Plasma Tau measurement could be useful for determining fitness for return to play.