

Ultrasensitive Tau Assay Enables Quantification of Neuronal Biomarker in Blood for the First Time

Introduction

Quanterix™ has developed a novel platform that enables the quantification of proteins present in blood and other body fluids at previously undetectable concentrations. The application of this Single Molecule Array (Simoa™) technology will provide medical researchers with an unprecedented tool for detecting low-abundance biomarkers and speed the development of a new generation of diagnostic products useful for early detection of disease. Unprecedented analytical sensitivity is the key differentiator of Quanterix's technology, made possible by its proprietary single molecule detection technology (to learn more about Simoa, see Whitepaper 1.0).

It has been well established that the neuronal protein tau has an important role in the diagnosis of Alzheimer's disease (AD). Currently, measuring neuronal proteins like tau requires cerebrospinal fluid (CSF) collection as their concentrations in peripheral circulation are below the detection limit of conventional assays. The necessity of an invasive collection method has thus limited use of CSF biomarkers. A means for measuring neuronal proteins in blood would dramatically influence diagnosis of neurodegenerative disease and other brain injuries by eliminating the need for invasive, expensive, time-consuming procedures. Until recently, efforts to provide sensitive and reliable tau measurements in serum and plasma have met with little success.^{1,2}

Method

The Simoa tau assay developed at Quanterix uses a combination of antibodies that reacts with both normal and phosphorylated epitopes in the midregion of the molecule, making the assay specific for all tau isoforms. The limit of detection of the assay is 0.02 pg/mL, which is over 1000-fold more sensitive than

conventional immunoassays (generally 30–60 pg/mL). The assay is based on digital array technology and uses the Tau5 monoclonal antibody for capture (Covance, Princeton, NJ, USA) and HT7 and BT2 monoclonal antibodies for detection (Pierce, now Thermo Fisher Scientific Inc., Waltham, MA, USA). The calibrator used is recombinant tau 381 (EMD Millipore Corporation, Billerica, MA). Figure 1 depicts a representative dose-response curve.

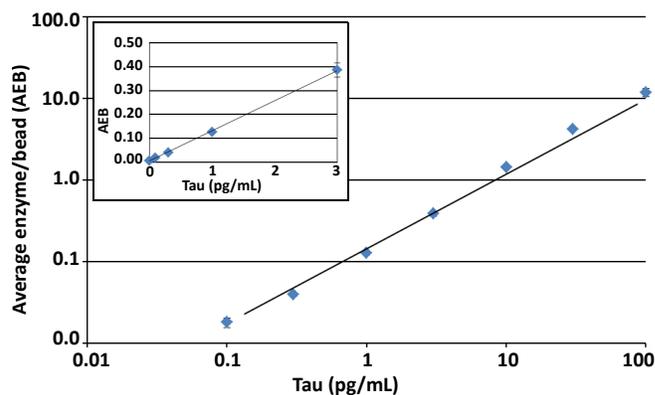


Figure 1 Typical tau calibration curve. The Y-axis refers to the average number of enzymes per individual microbead captured in the microwells of the array. Each labeled immuno-complex corresponds to a single molecule of tau.

Example 1, Brain Ischemia: Serum Tau Levels Predict Neurological Outcome after Hypoxic Brain Injury from Cardiac Arrest

Objectively measuring the severity of brain injury remains a significant unmet clinical need. Although clinical rating scales such as the Glasgow Coma Scale are useful for grading injury severity, and neuroimaging techniques are useful for identifying the nature and location of the injury, they have limited ability to predict short- and long-term outcome. Specific serum biomarkers could expedite diagnosis in sedated or

unconscious patients prior to neuroimaging, as well as stratify brain injury for targeted intervention. The potential usefulness of blood biomarkers for brain injury assessment, including hypoxic brain injury, has been studied over the last decade. Potential movement of elevated CSF tau across the blood-brain barrier presents a possibility that measurements of tau in blood could provide a convenient peripheral window into brain/CSF status.³

Twenty-five men and women aged 25 to 85 years (mean, 62 years) in cardiac arrest were resuscitated, then serial blood samples were collected within 6 hours following cardiac arrest. Time-dependent elevations of serum tau were observed in all patients (Fig. 2). Tau appearance estimated by area-under-the-curve (AUC) exhibited a statistically significant association with 6-month patient outcome ($p < 0.01$). For many patients with poor outcome, tau appeared in one or

long-term outcome than that of the first. Serial measurements of serum tau by Simoa were highly predictive of neurological outcome after 6 months, predicting poor and good outcomes with 91% sensitivity and 100% specificity, respectively.⁴ This study represented the first high-sensitivity longitudinal examination of serum tau after acute hypoxic brain injury. It is also the first study relating serum tau to hypoxic brain damage assessed by cerebral performance.

Example 2, Alzheimer's Disease: Plasma Tau Levels Are Increased in AD Patients

In clinical practice, a diagnosis of AD is primarily made based on clinical features, results of neurological and neuropsychological tests, and by exclusion of other causes of dementia, including vascular and frontotemporal dementia or other neurological diseases and conditions such as Parkinson's and Lewy body disease. Due to the clinical heterogeneity of AD, diagnosis remains uncertain until a post-mortem histopathological exam can be performed. The accuracy of clinical diagnosis among experienced investigators at leading medical centers is approximately 80% to 90%, and even lower at the primary care level, suggesting that improved methods for accurate diagnosis are needed. Guidelines have been proposed for what constitutes a useful biochemical marker for AD.⁵

A well-characterized biomarker that satisfies these guidelines should be able to detect fundamental neuropathology and be validated in autopsy-confirmed AD cases. Biomarker sensitivity should exceed 85%, and specificity should be higher than 80% for differentiating AD from other dementias. The ideal marker should also be reliable and reproducible across many laboratories, and the test should be noninvasive, simple to perform, and inexpensive. The ability to identify AD at the earliest stage of the disease before cognitive symptoms are identified is another important consideration in establishing biomarker value.

AD is characterized histopathologically by the presence of extracellular deposits of amyloid beta protein (A β)

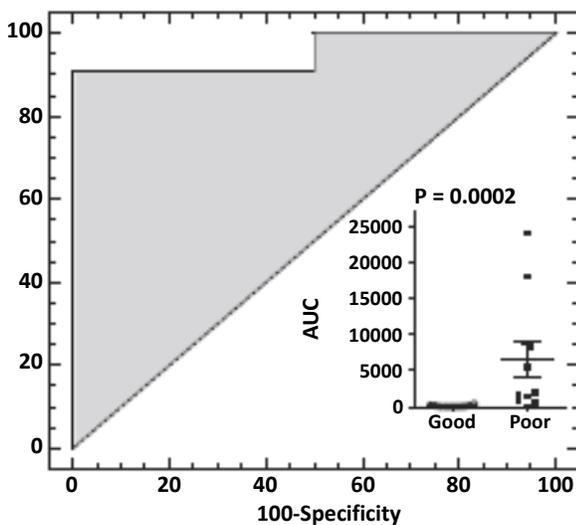


Figure 2 Tau AUC and associated ROC curves for the secondary tau peak only. “Good” and “Poor” refer to 6-month outcome by Cerebral Performance Category assessment.

both of two major elevation peaks, the first occurring soon after patient outcome and the second appearing days later. To evaluate the significance of the initial and delayed tau peaks for outcome, AUCs were calculated for the first 24 hours, the full time course, and the delayed peak only. The magnitude of the second peak appeared to be of somewhat greater significance for

that form plaques and intracellular accumulation of hyperphosphorylated and aggregated tau protein, which forms neurofibrillary tangles. Because the A β and tau proteins have been associated with histopathologically confirmed disease, there is great interest in these analytes. Although studies have been performed to assess A β 40 and A β 42 levels in serum or plasma, no studies have been able to evaluate levels of tau because current assays lack sufficient sensitivity to permit detection of this biomarker. The development of an ultrasensitive test could address this issue by allowing the diagnostic value of tau to be evaluated in blood for the first time.

Using the Simoa tau assay, the association of plasma tau levels with AD was assessed in a cross sectional study of 54 AD patients, 75 patients with mild cognitive impairment (MCI), and 25 cognitively normal controls.⁶ Tau levels were significantly higher in AD patients compared with both controls and MCI patients (Fig. 3), and MCI patients who developed AD during follow-up had tau levels similar to those of patients with stable MCI and cognitively normal controls. While plasma tau levels are elevated in AD, the overlap across diagnostic groups appears to limit tau's utility as a stand-alone screening test. Additionally, normal plasma levels of tau in the MCI stage of AD indicate that tau is a late marker, requiring substantial injury before increasing to abnormal levels. There was no correlation between tau levels in plasma and CSF in

any diagnostic group, suggesting that steady-state concentrations in these two body fluids are differentially regulated.

Example 3, Traumatic Brain Injury: Olympic Boxing Is Associated with Elevated Plasma Tau Levels

Traumatic brain injury, particularly mild traumatic brain injury (mTBI), is a common occurrence in athletes performing contact sports and in military personnel deployed in combat. Although overt symptoms of mTBI/concussion resolve rapidly in the majority of individuals, the overall burden of mTBI in terms of cognitive and behavioral impairments may be substantially underestimated. Identifying patient populations at risk of developing long-term consequences of TBI is an unmet diagnostic and prognostic need. Practical methods to accurately and reliably discriminate between mTBI that is or is not associated with clinically significant underlying brain damage are limited. Head CT scan is currently the initial diagnostic test of choice. However, it is limited by its inability to detect small contusions, white matter shearing, axonal injury, and small subacute hemorrhages. Advances in MRI technology have improved the ability to detect subtle brain injury, but high costs and limited access restricts clinical utility. There is an unmet clinical need for a way to measure circulating biomarkers that can detect

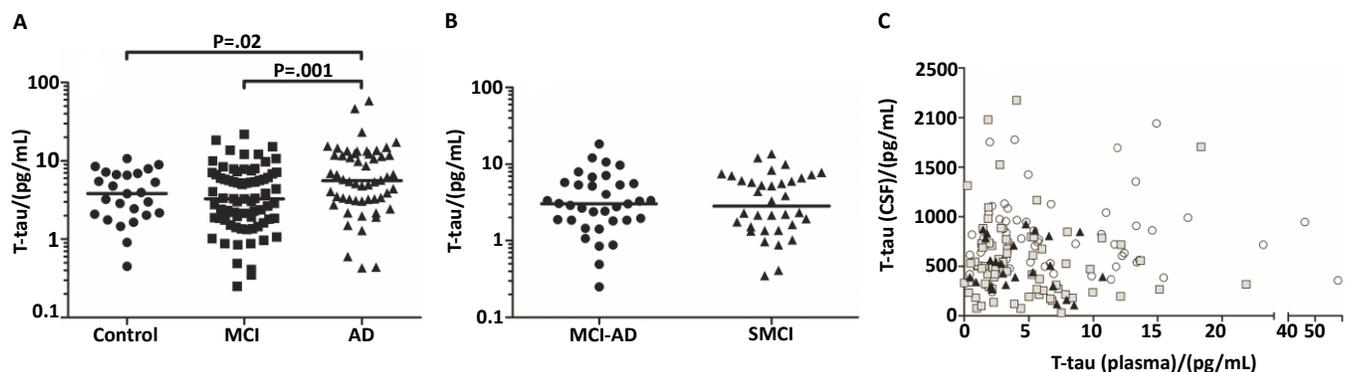


Figure 3 Elevated tau levels in plasma from patients with AD. A, Plasma levels of tau are elevated in patients with AD compared with cognitively normal controls and patients with MCI. B, MCI patients who developed AD (MCI-AD) during follow-up had baseline tau levels similar to those of patients with stable MCI (SMCI). C, There was no correlation between tau levels in plasma and CSF in any diagnostic group. Open circles, gray squares, and black triangles represent AD, MCI, and controls, respectively.

mTBI and identify patients who need further evaluation and therapy. CSF biomarkers of brain injury have already been established, including proteins that indicate neuroinflammation and neuronal, axonal, and glial damage. However, CSF is not a readily accessible biological fluid, particularly for individuals with mild and moderate TBI, and currently available assays lack sensitivity to detect neuronal biomarkers in peripheral fluids.

Boxers represent a population with an increased risk of TBI caused by minimal, mild, or moderate/severe damage. TBI may also be caused by the cumulative effect of subconcussive translational and rotational punches to the head. These types of forces may result in cortical damage and diffuse axonal injury. In addition, there is growing evidence that recurrent episodes of head injury may lead to chronic TBI. Thirty Olympic (amateur) boxers competing in at least 47 bouts were compared to 25 controls.⁷ Blood was collected from the controls at one occasion and from the boxers within 1–6 days after a bout and after a rest period of at least 14 days. Tau concentration in plasma was determined using the Simoa digital assay.

Plasma tau was significantly increased in the boxers after a bout ($2.46 \text{ pg/mL} \pm 5.1$) compared to controls ($0.79 \text{ pg/mL} \pm 0.96$) (Fig. 4). Plasma tau decreased significantly in the boxers after a resting period compared to after a bout ($p < 0.01$). No significant difference was found between controls and boxers at follow-up. Results indicate that boxing that may lead to axonal injuries can be diagnosed with a blood test. This study demonstrates that Olympic boxing and repetitive minimal head trauma are associated with elevations of plasma tau, even in the absence of symptoms of concussion or minimal TBI, and that Simoa's sensitivity offers a new tool for measuring and studying the relationship between neurological biomarkers and clinical presentation.

Conclusion

These data demonstrated that Simoa is the first platform capable of directly measuring the appearance of

tau in human serum and plasma. The reliable detection and quantification of tau in blood is of potential clinical significance for several important applications. Further research using Simoa may demonstrate the ability to measure other brain biomarkers readily in blood, providing new insights for diagnosis, monitoring, and treatment of several neurological conditions.

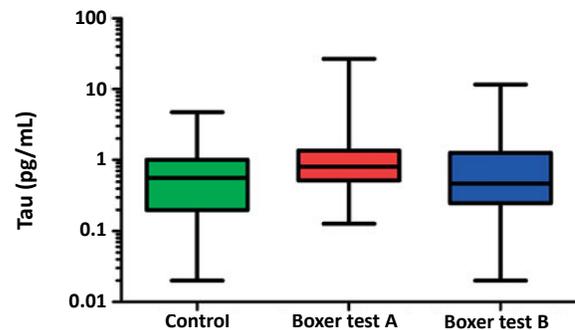


Figure 4 Plasma tau is significantly elevated in boxers after a bout compared to controls. Peripheral blood was collected from the controls once. The boxers were tested 1–6 days after a bout (A) and after a rest period without exposure to bouts or training with blows to the head for at least 14 days (B).

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Tau assay kit catalog number: 100113

For more information:

www.quanterix.com
sales@quantarix.com
617-301-9400 ext. 495