# Serum Tau is predictive of 6-month neurological outcome following cardiac arrest

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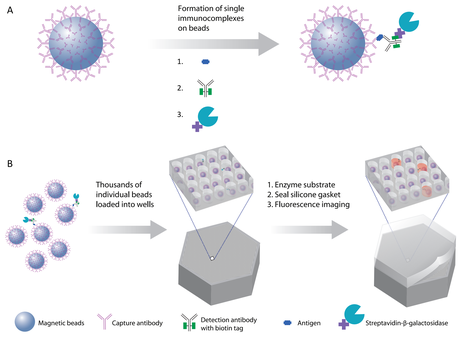
Annual Meeting of the Society for Neuroscience, Washington, D.C. November 12-16, 2011

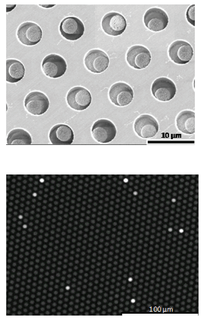
## Background

Tau, a microtubule associated protein, plays an important role in the assembly of tubulin monomers into microtubules and maintaining the cytoskeleton and axonal transport. The presence of tau in cerebrospinal fluid (CSF), serum or plasma is thought to represent neuronal damage from physical trauma or apoptotic death of neurons. During ischemic injury from cardiac arrest, cell death could occur from the initial hypoxic insult as well as an apoptotic cascade resulting in delayed neuronal death. The measurement of tau in CSF has been well documented, but the presence and measurement of tau in human blood components has been difficult due to inadequate sensitivity of current assay methods. A direct link between acute oxygen deprivation and the appearance of tau in human periphery has not been previously studied. We employed a new technology (Single Molecule Arrays, SiMoA) capable of ultrasensitive protein measurements to look for changes in serum tau in patients following cardiac arrest and resuscitation, and to correlate these changes to 6-month neurological outcome.

## Method

26 unconscious patients with cardiac arrest were resuscitated with restoration of spontaneous circulation (ROSC). Serial blood samples were collected within 6h after cardiac arrest, and continued at intervals from 1-108h. Inclusion criteria included age, systolic BP >80mmHg after ROSC, and a Glasgow Coma Scale ≤7. Patient outcome was assessed in accordance with the Glasgow-Pittsburgh cerebral performance category (CPC) scale at discharge from the intensive care unit and 6 months later. Serum aliquots were frozen until assay. Samples were measured in triplicate by SiMoA Tau assay, which has a limit of detection of 0.02 pg/mL.



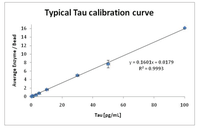



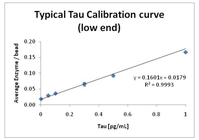

**Tau assay based on arrays of femtoliter wells.** (A) Capturing and labeling single protein molecules on beads using standard ELISA reagents. Assay components are: 1) anti-Tau coated paramagnetic microbeads; 2) biotinylated anti-Tau detector antibody; 3) conjugate of streptavidin--galactosidase. (B) Loading of beads into femtoliter well arrays for isolation and detection of single molecules. (C) SEM image of a small section of a femtoliter well array after bead loading. 2.7-µm-diam. beads were loaded into an array of wells with diameters of 4.5 µm and depths of 3.25 µm. (D) Fluorescence image of a small section of the femtoliter well array after signals from single enzymes are generated.

### SiMoA Assay Sequence

- A bead-based ELISA is performed using anti-Tau coated paramagnetic microbeads for solid phase capture and magnets to collect beads during washing.
- Microbeads from the ELISA are loaded onto arrays of femtoliter volume wells and sealed with enzyme substrate (resorufin -D-galactopyranoside, RDG).
- Arrays are interrogated for fluorescent product. A rising level of fluorescence in the wells indicates the presence of labeled Tau molecules.
- Positive wells are counted as % active wells. At low Tau concentrations the wells contain either one or zero Tau molecules (per Poisson statistics). This results in a digital signal of either "positive" or "negative".
- The average intensity of the signal is converted to "average enzymes per bead" (AEB), which reflects the average number of labeled Tau immunocomplexes bound to each microbead within the array.
- When every bead has at least one enzyme (higher Tau concentrations), analog fluorescence intensity is converted to AEB based on measurements in the digital, lower Tau range, extending dynamic range.
- Unknown Tau concentrations are interpolated from a standard curve of AEB vs. known Tau.

### Assay Dose-Response




**Assay Dose-response:** Representative dose response of serial dilutions of Tau. Y-axis refers to the average number of label enzymes per individual microbead captured in the microwells of the array. Each labeled immunocomplex corresponds to a single molecule of Tau.

## Results

### Hypoxia induced changes in serum Tau

Time-dependent elevations of serum tau were observed in all patients. Tau appearance as 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 both of two major elevation peaks, the first occurring soon after cardiac arrest, and the second appearing days later. The magnitude of the second peak appeared to be of somewhat greater significance for long-term outcome than the first.

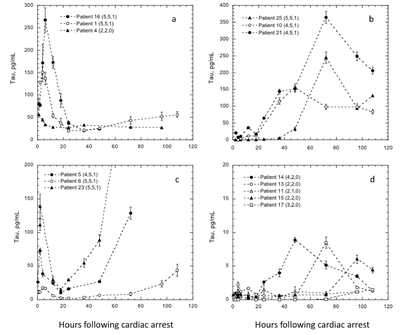



**Figure 1.** Longitudinal serum tau concentrations following resuscitation from cardiac arrest. CPC scores are listed for each patient in the legends. The first two numbers correspond to CPC assessments upon discharge from the ICU and six months later. The third number represents overall 6-month outcome assessment (1=poor, 0=good). Fig 1a-c depicts representative groupings of patients exhibiting different profiles of tau elevation: initial 24-hour peaks (**Fig 1a**), delayed peaks (**Fig 1b**), and both (**Fig 1c**). **Fig 1d** depicts representative profiles from patients with good outcome. Note difference in scales. Error bars depict SD of triplicate measurements.

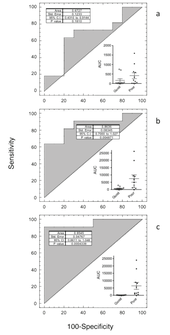



**Figure 2.** Tau AUC results (**Table 1**) and associated ROC curves across the first 24 hours (**Fig 2a**), all serial samplings (**Fig 2b**), and the secondary tau peak only (**Fig 2c**). "Good" and "Poor" refers to 6-month outcome. Error bars in insets depict standard error of the means.

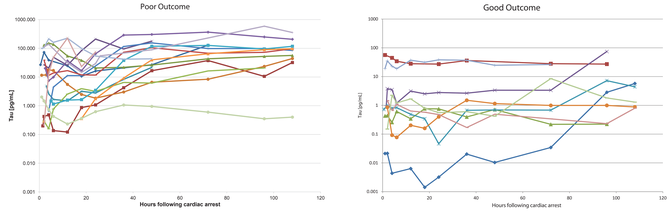



**Figure 3.** Overlay plots of longitudinal serum Tau concentrations calculated from SiMoA calibration curve. "Poor" outcome (**Fig.3a**). "Good" outcome (**Fig.3b**). Individual points depict average of triplicate measurements. Error bars intentionally left off for clarity of graphs.

| Patient | AUC </= 24 hr | | AUC all | | AUC 2nd peak only | |
|---|---|---|---|---|---|---|
| | Good | Poor | Good | Poor | Good | Poor |
| 2 | | 1590 | | 4759 | | 1440 |
| 3 | 708.9 | | 2145 | | 0 | |
| 4 | 748.3 | | 2861 | | 154.2 | |
| 5 | | 625.2 | | 3007 | | 1890 |
| 6 | | 179.9 | | 1232 | | 897.5 |
| 7 | 4.72 | | 92.86 | | 54.5 | |
| 8 | | 11.19 | | 1646 | | 1545 |
| 9 | 59 | | 1141 | | 0 | |
| 10 | | 109.7 | | 9336 | | 8206 |
| 11 | 14.63 | | 84.56 | | 0 | |
| 12 | | 52.15 | | 848.2 | | 567.8 |
| 13 | 26.01 | | 288.5 | | 215.8 | |
| 14 | 12.93 | | 431.5 | | 393 | |
| 15 | 12.31 | | 184.4 | | 168.2 | |
| 17 | 0.02 | | 30.68 | | 30.9 | |
| 18 | 15.78 | | 46.62 | | 0 | |
| 20 | | 334.6 | | 6827 | | 5511 |
| 21 | | 580.8 | | 19875 | | 18051 |
| 23 | | 1039 | | 26150 | | 23935 |
| 24 | | 12.92 | | 72.9 | | 35.94 |
| 25 | | 9.37 | | 8987 | | 8842 |
| AVG | 160.3 | 413.2 | 730.6 | 7521.8 | 101.7 | 6447.4 |
| *P value* | 0.0963 | | 0.0027 | | 0.0002 | |

**Table 1.** Characteristics of serum tau elevation profiles from resuscitated survivors of cardiac arrest during the first 96-108 hours following admission to the ICU. The data were sorted on the basis of good or poor 6-month cerebral outcome and compared by Mann-Whitney test. Four patients (all with good outcome) exhibited no discernable secondary peaks.

## Conclusion

These data are the first to directly measure the effects of oxygen deprivation on the appearance of tau in human periphery. The kinetic profiles could be related to acute initial hypoxic injury followed by apoptotic neuronal death and/or damage associated with cerebral swelling. Serum tau elevation was found to exhibit a strong association with 6-month neurological outcome.

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