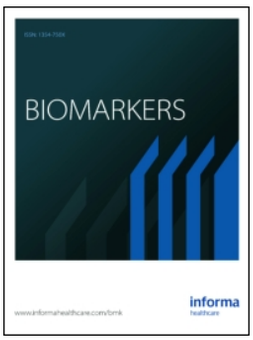

Biomarkers

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Serum tau fragments as predictors of death or poor neurological outcome after out-of-hospital cardiac arrest

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Disclosure of interest

HZ has served at scientific advisory boards for Roche Diagnostics, Samumed, Wave and CogRx, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (all outside submitted work)

The remaining authors report no conflict of interest.

Running header

Serum tau fragments after cardiac arrest

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Clinical significance

- We examined the prognostic value of two biomarkers, tau-A and tau-C, which are enzymatically produced fragments of the neuron-specific protein tau. Tau is a good prognostic marker of brain injury in patients, who are comatose after out-of-hospital cardiac arrest.

- The two tau-fragments, tau-A and tau-C are easy to measure in peripheral blood and theoretically passes the blood-brain-barrier easier as compared to whole tau. However, the prognostic value of tau-A and-tau-C is poor, and these fragments should not be used for prognostication after out-of-hospital cardiac arrest.

Abstract

Background

Anoxic brain injury is the primary cause of death after resuscitation from out-of-hospital cardiac arrest (OHCA) and prognostication is challenging. The aim of this study was to evaluate the potential of two fragments of tau as serum biomarkers for neurological outcome.

Methods

Single-center sub-study of 171 patients included in the Target Temperature Management (TTM) Trial randomly assigned to TTM at 33°C or TTM at 36°C for 24 hours after OHCA. Fragments (tau-A and tau-C) of the neuronal protein tau were measured in serum 24, 48 and 72 hours after OHCA. The primary endpoint was neurological outcome.

Results

Median (quartile 1 – quartile 3) tau-A (ng/ml) values were 58 (43-71) versus 51 (43-67), 72 (57-84) versus 71 (59-82) and 76 (61-92) versus 75 (64-89) for good versus unfavorable outcome at 24, 48, and 72 h, respectively (p_{group} = 0.95). Median tau C (ng/ml) values were 38 (29-50) versus 36 (29-49), 49 (38-58) versus 48 (33-59), and 48 (39-59) versus 48 (36-62) (p_{group} = 0.95). Tau-A and tau-C did not predict neurological outcome (area under the receiver-operating curve at 48 hours; tau-A: 0.51 and tau-C: 0.51).

Conclusions

Serum levels of tau fragments were unable to predict neurological outcome after OHCA.

Key words

Serum biomarkers; tau fragments; neurological outcome; Out-of-Hospital Cardiac Arrest; prognosis

Introduction

In the initial phase after resuscitated out-of-hospital cardiac arrest (OHCA), patients are often comatose and treated with targeted temperature management (TTM) (Nielsen *et al.*, 2013a, Hassager *et al.*, 2018). The prognosis is uncertain, and reliable methods of prognostication in the first days after resuscitation are needed (Hassager *et al.*, 2018). There is an ongoing search for reliable blood-borne biomarkers of brain injury to predict outcome after initial resuscitation (Stammet *et al.*, 2015, Bro-Jeppesen *et al.*, 2016b, Mattsson *et al.*, 2017, Stammet *et al.*, 2017). An ideal biomarker would differentiate with high precision between patients with severe anoxic brain injury and patients with favorable outcome. Currently several validated blood biomarkers of brain injury are known, including neuron-specific enolase (NSE), neurofilament and total tau (T-tau) (Stammet *et al.*, 2015, Mattsson *et al.*, 2017, Moseby-Knappe *et al.*, 2018). T-tau is a strong predictor of neurological outcome after OHCA; an elevation in serum T-tau levels has been shown to be a better predictor of poor outcome after OHCA than NSE (Randall *et al.*, 2013, Mattsson *et al.*, 2017). The tau protein undergoes many post-translational modifications, and these generate several different neo-epitope fragments (Hanger and Wray, 2010, Wang *et al.*, 2012). A widely studied fragment of tau is the caspase-3-generated tau-C, which reflects initiation of neuronal cell death (Behl, 2000, Hanger and Wray, 2010, Henriksen *et al.*, 2015). Another reported tau fragment is the ADAM10-generated tau-A (Henriksen *et al.*, 2013). Serum-levels of tau-A and tau-C are associated with Alzheimer's disease (Inekci *et al.*, 2015) and severity of sports-related concussion (Shahim *et al.*, 2016) and furthermore, they have been reported to correlate inversely with cognitive function (Henriksen *et al.*, 2013). Tau fragments may have the advantage of passing the blood-brain barrier due to their smaller size and be present in higher concentrations in the circulation when compared to intact protein (T-tau) (Wang *et al.*, 2012). Another clinical advantage of tau fragments,

is that tau-A and tau-C can be measured with more simple techniques (solid phase competitive enzyme linked immunosorbent assays (ELISAs)) in contrast to T-tau, which is measured using the ultrasensitive single molecule array (Simoa) technique (Henriksen *et al.*, 2013, Shahim *et al.*, 2014, Henriksen *et al.*, 2015, Shahim *et al.*, 2016).

We hypothesized that serum-levels of tau-A and tau-C could predict neurological function after OHCA. Accordingly, we assessed the association between tau-A and tau-C with death and neurological outcome in comatose patients resuscitated after OHCA. Second, we assessed the effect of TTM at 33°C (TTM33) versus 36°C (TTM36) on tau-A and tau-C concentrations.

Materials and methods

Study Design

This study is a single-center, prospective, observational sub-study of 171 patients enrolled at Copenhagen University hospital, Rigshospitalet in the TTM-Trial (November 2010 to July 2013), which compared two temperature regimens in comatose adult patients admitted after OHCA of presumed cardiac cause (Nielsen *et al.*, 2013a). Patients were excluded if unwitnessed asystole was the primary rhythm, the time between ROSC and screening exceeded 4 hours, if an intracranial bleeding was suspected or confirmed or temperature on admission was less than 30°C. Patients were randomly assigned 1:1 to TTM33 or TTM36 for 24 hours after cardiac arrest. Investigators were unblinded for temperature allocation. The TTM-trial design, the statistical analysis plan, and the main results have been published previously (Nielsen *et al.*, 2012, Nielsen *et al.*, 2013a, Nielsen *et al.*, 2013b). All patients were sedated, intubated and mechanically ventilated and active cooling was initiated immediately after randomization. Target temperature was induced and maintained for 28 hours after ROSC, followed by active rewarming of no more than 0.5°C/hour to 37°C. Sedation was discontinued and the patients could recover spontaneously after 36 hours. We assessed the

association between tau fragments and mortality and neurological outcome after 180 days. Neurological outcome was dichotomized according to the cerebral performance category (CPC) scale into good or poor outcome (CPC 1-2 = good; CPC = 3-5 = poor). The CPC scale ranges from 1 to 5; 1 represents good cerebral performance or minor disability; 2, moderate disability; and 3, severe disability; 4, coma or vegetative state; and 5, death. Prehospital cardiac arrest data in addition to patient data and demographics were collected in accordance with the Utstein guidelines (Langhelle *et al.*, 2005). The Ethics Committee of the Capital Region of Copenhagen approved the main study protocol (H-1-2010-059) and written informed consent was obtained from patients' next of kin and general practitioner in all cases and from patients regaining consciousness after cardiac arrest. The TTM-trial is registered at ClinicalTrials.gov (identifier: NCT01020916) and complies with the Declaration of Helsinki. Good Clinical Practice was followed. All patients were actively treated in minimum 72 hours after rewarming (108 hours after cardiac arrest). At this time point, neurologic prognostication for all unconscious patients was performed by a physician, who made a recommendation on level-of-care (continue or "do not escalate" or "withhold active intensive care"). Neurological prognostication as well as withdrawal of life-supporting therapies were standardized and reported according to the trial protocol and results from prognostication have previously been published (Nielsen *et al.*, 2012, Dragancea *et al.*, 2015). In all cases there was adherence to this protocol.

Biomarkers

Serum blood samples were collected at 0, 24, 48, and 72 (admission, day 1, 2 and 3) hours after admission. All samples were pre-analytically centrifuged, aliquoted, and frozen to -80°C. In serum, ELISAs were used to assess the neo-epitope fragments of tau. Both assays have previously been described in detail elsewhere (Henriksen *et al.*, 2015). Each neo-epitope constitutes an amino acid sequence that has been generated by enzymatic cleavage. The assays used are based on mouse

monoclonal antibodies and are specific for caspase-3 and ADAM10-generated neo-epitope fragments of tau protein. The tau-A assay detects the ADAM10-generated cleavage site of tau with an intra-assay coefficient of variation (CV) of 5.8% and an inter-assay CV of 12.6%. The tau-C assay detects the caspase-3-generated cleavage site of tau with an intra-assay CV of 5.7% and an inter-assay CV% of 15%. The antibodies only react specifically with the cleaved protein and not with intact protein (T-tau) and, therefore, only detect tau fragments. The lower limits of quantification (LLOQ) for tau-A and tau-C assays were determined as 11.9 ng/mL and 10.4 ng/mL, respectively. If measured values fell below the LLOQ, this value was assigned to the sample measurement (Henriksen *et al.*, 2013, Henriksen *et al.*, 2015). Data regarding T-tau for the entire TTM trial (n=686) has previously been published (Mattsson *et al.*, 2017). T-tau was measured in plasma with the Human Total Tau kit (Quanterix, Lexington, MA) on the Simoa HD-1 analyzer. This assay uses a monoclonal antibody that reacts with an epitope in the mid-region of all the isoforms of tau in addition to a detection antibody reacting with a linear epitope in the N-terminal region of tau (Mattsson *et al.*, 2016). Biomarker data were not available to the treating physicians during the trial. C-reactive protein, leucocytes and hemoglobin was measured at the local biomedical laboratory and the highest daily value was reported.

Endpoints

The primary endpoint was good neurological outcome after 180 days. Secondary endpoint was 180-day mortality.

Statistics

Data is presented as mean and standard deviation (SD) or median and lower and upper quartile (interquartile range (IQR)) according to data-distribution. Associations between tau fragments and baseline variables were tested by linear regression. Spearman's rho (r) correlation coefficients were

used to estimate correlations between variables. Logarithmic transformation of variables was performed as appropriate before analysis to reduce skewness. Differences between groups were assessed by Student's t-test or Wilcoxon two-sample test. Categorical variables were tested with the chi-square test. Logistic-regression analysis was used to assess the diagnostic value of tau-A and tau-C in relation to neurological outcome. Results are reported as odds ratios (OR). Receiver-operator characteristic (ROC) curves were computed to assess the discriminating power of the biomarkers. Cox regression was used to assess association between tau-fragments and 180-day mortality. Results are reported as hazard ratios (HR) with 95% confidence intervals. Kaplan–Meier plots for quartiles of tau fragments was created. Differences in biomarkers measured at 24, 48 and 72 hours between patients with good or poor neurological outcome were assessed by repeated-measurements mixed models with an unstructured covariance structure. Neurological outcome (good/poor) and time points were fixed effects. The interaction term of neurological outcome with time was included. Before analysis in the mixed models, assumptions were checked and met in all cases. We did not adjust for baseline differences. Missing values of both tau A and tau C were below 5% at baseline. A two-sided p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the SAS statistical software, version 9.4 (SAS Institute, Cary, NC).

Results

A total of 171 OHCA-patients were included at the Copenhagen University hospital. At least one value of tau-A or tau-C was available from 165 patients (96%), defining the tau fragment study cohort (Figure 1). The mean age was 62 years, 88% were male and 87% had ventricular fibrillation as initial rhythm. Detailed demographics, prehospital data and comorbidities are shown in Table 1. Patients with a good neurological outcome were younger, had more often a witnessed arrest and an initial shockable rhythm, had shorter time to ROSC and had lower initial lactate (Table 1). No

differences between baseline demographics or comorbidities were seen between TTM33 and TTM36.

Increased levels of tau-A were associated with male sex (24 hours (p=0.003), 48 hours (p=0.02), 72 hours (p=0.02)) and bystander defibrillation (24 hours (p=ns), 48 hours (p=0.008), 72 hours (p=0.006)), however tau-C was not associated with any of the demographic variables or comorbidities. Both tau-A and tau-C increased over time (Tau-A: $p_{time}<0.0001$; Tau-C: $p_{time}<0.0001$) with the main increase between admission and 48 hours (Figure 2). No differences in tau-A or tau-C were seen between temperature groups at any time point (Table 2).

Prognostic implications of tau fragments

After 180 days, 103 (62%) patients survived with a good neurological outcome. No differences in tau-A or tau-C were seen between patients with good and poor neurological outcomes for any time point: Median (IQR) tau-A values were 58 (43-71) ng/ml versus 51 (43-67) ng/ml, 72 (57-84) ng/ml versus 71 (59-82) ng/ml, and 76 (61-92) ng/ml versus 75 (64-89) ng/ml for good versus poor outcome at 24, 48, and 72 h, respectively (p_{group} = 0.95). Median tau-C values were 38 (29-50) ng/ml versus 36 (29-49) ng/ml, 49 (38-58) ng/ml versus 48 (33-59) ng/ml, and 48 (39-59) ng/ml versus 48 (36-62) ng/ml for good versus poor outcome at 24, 48, and 72 h, respectively (p_{group} = 0.9) (Figure 2).

Levels of tau-A at day 1 (HR: 0.99 [0.98-1.01], p=0.57), and day 2 (HR: 1.00 [0.99-1.02], p=0.78) and levels of tau-C at day 1 (HR: 0.99 [0.98-1.02], p=0.65) and day 2 (HR: 0.99 [0.98-1.02], p=0.79) were not associated with 180-day mortality. Figure 3 shows 180-day mortality for tau-fragments stratified into quartiles. Levels of T-tau at day 1 (HR: 1.59 [1.38-1.82], p<0.0001), and day 2 (HR: 1.58 [1.42-1.76], p<0.0001) were significantly associated with 180-day mortality in this cohort. As previously, no interaction between temperature-group and levels of tau-A at day 1

($p_{interaction}$ = 0.33) and day 2 ($p_{interaction}$ = 0.83) and tau-C at day 1 ($p_{interaction}$ = 0.37) and day 2 ($p_{interaction}$ = 0.33) were found for mortality.

Tau-A and tau-C were not associated with poor neurological outcome at any time point (tau-A: day 1, OR: 0.99 [0.98-1.01], p=0.75, day 2: OR: 1.00 [0.99-1.02], p=0.76, day 3: OR: 1.00 [0.99-1.02], p=0.74. Tau-C: day 1, OR: 0.99 [0.98-1.02], p=0.84, day 2: OR: 0.99 [0.98-1.02], p=0.81, day 3: OR: 1.00 [0.98-1.02], p=0.84). Furthermore, as illustrated in Figure 4, ROC-curves showed that tau-A and tau-C levels were poor predictors of neurological outcome at day 1 (tau-A: AUC: 0.55 (95% CI: 0.45–0.64, p = 0.64), tau-C: AUC: 0.53 (95% CI: 0.43–0.63, p = 0.74), day 2 (tau-A: AUC: 0.51 (95% CI: 0.41–0.60, p = 0.81), tau-C: AUC: 0.51 (95% CI: 0.41–0.61, p = 0.86), day 3 (tau-A: AUC: 0.52 (95% CI: 0.42–0.63, p = 0.80), tau-C: AUC: 0.51 (95% CI: 0.41–0.61, p = 0.89).

T-tau was associated with poor neurological outcome (T-tau: day 1, OR: 2.44 [1.77-3.37], p<0.0001, day 2: OR: 3.54 [2.33-5.37], p<0.0001, day 3: OR: 3.76 [2.39-5.93], p<0.0001) and was a good predictor of neurological outcome in this cohort (day 1; AUC: 0.84 (95% CI: 0.78–0.91, p = 0.17), day 2; AUC: 0.90 (95% CI: 0.85–0.95, p = 0.0003), day 3; AUC: 0.90 (95% CI: 0.84–0.96, p < 0.0001)).

Correlation between Tau fragments and other biomarkers

Levels of tau-A correlated with tau-C at admission (r=0.61), day 1 (r = 0.66), day 2 (r = 0.7) and day 3 (r = 0.67) after OHCA, all p < 0.0001. Tau-A did not correlate with T-tau at day 1 (r = 0.05; p=0.49), day 2 (r = 0.06; p=0.51) or day 3 (r = 0.09; p=0.27). A similar pattern was seen for T-tau and tau-C at day 1 (r=0.15; p=0.06), day 2 (r=0.04; p=0.62) and day 3 (r=0.05; p=0.58) (table 2).

Tau-A correlated with C-reactive protein at day 1 (r=0.39; p<0.0001), day 2 (r=0.37; p<0.0001) and day 3 (r=0.28; p=0.0007). Even closer correlated with C-reactive protein was tau-C at day 1 (r=0.50; p<0.0001), day 2 (r=0.48; p<0.0001) and day 3 (r=0.42; p<0.0001). T-tau, however, was

not correlated with C-reactive protein at day 1 (r=0.14; p=0.06), day 2 (r=0.17; p=0.06) or day 3 (r=0.06; p=0.46). At day 1, tau-A and tau-C were furthermore correlated with procalcitonin (tau-A: p=0.04; tau-C: p=0.003) and hemoglobin (tau-A: p=0.03; tau-C: p=0.03), but not with leukocytes (Table 3).

Discussion

To our knowledge, this is the first prospective and blinded study of tau fragments in consecutively admitted, comatose OHCA-patients and the largest clinical study of tau fragments overall. We report, that serum tau-A and tau-C concentrations at admission and after 24, 48 and 72 hours were not able to predict neurological outcome, making these novel biomarkers non-useful in the clinical setting as prognostic factors after OHCA. Level of target temperature did not influence concentrations or prognostic value of these biomarkers. Furthermore, the tau fragments correlated with each other but not with T-tau.

This is the first study to measure tau fragments in serum from resuscitated OHCA-patients. Serum T-tau has recently proven a more accurate predictor of poor outcome compared with serum NSE, which is currently the most used biochemical marker for prognostication after OHCA (Nolan *et al.*, 2015, Stammet *et al.*, 2015, Mattsson *et al.*, 2017). Tau-A and tau-C are measured using standard ELISA, in contrast to T-tau, which can only be measured using the ultrasensitive Simoa technique (Henriksen *et al.*, 2013, Shahim *et al.*, 2014, Shahim *et al.*, 2016, Mattsson *et al.*, 2017). It is unclear why levels of these tau fragments are easier to detect in peripheral blood than T-tau, but likely, due to their smaller size, they are transported across the blood-brain barrier more easily, which allows quantification by standard ELISA methods in serum. Tau fragments are made by enzymatic cleavage of whole tau proteins, which is assumed to originate from injured neurons. Therefore, the hypothesis has been made, that tau fragments measured in serum reflects the degree

of brain injury and is able to predict prognosis. Tau fragments have proved beneficial in previous studies where serum-levels of tau-A and tau-C were associated with Alzheimer's disease and severity of sports-related head trauma (Shahim *et al.*, 2014, Henriksen *et al.*, 2015, Inekci *et al.*, 2015, Shahim *et al.*, 2016). Furthermore, cognitive function has been shown to be inversely related with serum-levels of tau-A and tau-C,(Henriksen *et al.*, 2013) and tau-C levels were indicative of future dementia diagnosis in a study of over 5,500 elderly women (Neergaard *et al.*, 2018). In our study of resuscitated, comatose, OHCA patients, we have measured tau-A and tau-C at several time points after resuscitation and found no association between serum levels of the tau fragments and brain injury. In the OHCA population, T-tau has previously been associated with brain injury and neurological outcome (Mattsson *et al.*, 2017), but surprisingly, the finding of this study is that the tau fragments are not correlated with T-tau. The blood-brain barrier is injured after OHCA in some patients due to anoxic injury (Mussack *et al.*, 2001). We speculate that T-tau passes the blood-brain barrier in patients with anoxic brain injury, which results in much higher concentration in serum compared to patients without anoxic injury. Since tau fragments already can pass the intact blood brain barrier, they are not measured differently between patients with or without anoxic injury. This hypothesis can be investigated in future studies comparing tau concentration in serum with tau concentration in cerebrospinal fluid, which has not yet been done in OHCA patients, possibly because most cardiac arrest patients receive anticoagulation within the first hours after admission. Therefore, studies of cerebrospinal fluid after OHCA, should be performed in experimental animal models or in the few OHCA-patients, without indication for anticoagulation (ie. no suspicion of myocardial ischemia). Furthermore, OHCA-patients are different from the subjects investigated in previous studies of tau fragments in several ways. First, the previous conditions, like Alzheimer's disease and impaired cognitive function, develop over long time while OHCA results in hyper-acute anoxic brain injury (Laver *et al.*, 2004, Sekhon *et al.*, 2017, Hassager *et al.*, 2018), leading to a

higher and faster leak of T-tau from the brain. This may cause a bottleneck-effect of T-tau degradation if the caspase-3 and ADAM10 enzymes are saturated. This hypothesis is supported by the fact, that the variability (interquartile range) of tau-A and tau-C concentrations in our study is much lower compared to other biomarkers as T-tau and NSE as shown in figure 1; a limit in enzymatic T-tau degradation will result in a natural upper limit of the tau fragments in the days after OHCA. Furthermore, as seen in Figure 2, the concentration of T-tau seems to reach a peak after 48 hours, whereas the tau fragments do not. It is possibly, that a difference between good and poor neurological outcome could be identified by tau fragments later than 72 hours by investigating the time to return to normal levels. However, a possible prognostic value of tau fragments after so long time make them unsuitable for clinical use in OHCA patients. This study implicates that caution should be made, if tau fragments are used clinically in other acute situations, where brain injury happens over a short time period.

The systemic inflammatory response after OHCA results in a surge of systemic cytokines and dysfunction of various enzymes and organ systems (Nolan *et al.*, 2010, Bro-Jeppesen *et al.*, 2014, Bro-Jeppesen *et al.*, 2015a, Bro-Jeppesen *et al.*, 2015b, Johansson *et al.*, 2015, Bro-Jeppesen *et al.*, 2016a, Bro-Jeppesen *et al.*, 2016b). An experimental study has found a downregulation in caspase-3 mRNA in rats undergoing hypothermia after cardiac arrest,(Lu *et al.*, 2014) potentially resulting in impaired enzymatic breakdown of T-tau after OHCA. Since the prognostic value of T-tau is good even after 72 hours (Mattsson *et al.*, 2017), this could be the result of the impaired breakdown of this protein. In a study of sports-related concussion, tau-A levels at 1–12 h post-concussion were linearly related to T-tau levels assessed 1–12 h post-concussion, but not with tau-C levels (Shahim *et al.*, 2016). Tau fragments in our study correlated with biomarkers of inflammation such as C-reactive protein, suggesting that tau fragments interact with the immunologic and systemic inflammatory response after OHCA. Interactions of tau fragments with inflammation can be

investigated in future studies. The methods used in this study have been used and validated in numerous previous studies with great reliability (Henriksen *et al.*, 2013, Henriksen *et al.*, 2015, Inekci *et al.*, 2015, Shahim *et al.*, 2016, Neergaard *et al.*, 2018). We have showed that the two tau fragments increase as expected after OHCA in the same way as other biomarkers (Stammet *et al.*, 2015, Mattsson *et al.*, 2017), are correlated with each other and correlates with several other biomarkers in this study. These findings confirm that measurements are reliable even though the negative findings in this study is surprising.

Limitations

First, tau fragments may be susceptible to patient-specific mechanisms which are not yet fully understood. Secondary, it is possible that extra-cerebral sources of tau fragments exists, which are released after OHCA thus interfering with the prognostic value of tau fragments, as is seen with S100B after trauma (Stammet *et al.*, 2017). Third, the down-stream effects of whole-body ischemia and reperfusion injury following OHCA affects all cell types in the brain and the entire body. Therefore, other aspects of the post-cardiac arrest syndrome potentially affect the tau fragment measurements in ways we do not yet understand. Lastly, this was a single-center study and therefore our results cannot necessarily be extrapolated to other centers.

Conclusions

This study shows that the enzymatically produced fragments of tau, tau-A and tau-C, are not associated with outcome after resuscitated OHCA. Tau fragments were, however, associated with levels of systemic inflammation, assessed by C-reactive protein and procalcitonin. The level of target temperature did not influence concentrations of tau fragments. Further studies regarding how tau is proteolytically processed in blood in other patient groups, as well as in relation to inflammation, could potentially increase the interpretability of tau fragments as biomarkers.

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Figure captions (as a list)

Figure 1: Flow chart depicting the flow of patients included in the Target Temperature Management (TTM) trial at Copenhagen University Hospital Rigshospitalet. CPC = Cerebral performance category.

Figure 2: Serum concentrations of tau fragments and total tau stratified into groups with good neurological outcome (cerebral performance category=1-2) and poor neurological outcome (cerebral performance category=3-5) after 180 days. Curves show median values with error bars representing interquartile range. Reported p-values are tested after log-transformation in a mixed-model. Data in panel C has been published previously as part of a study assessing total tau in the entire TTM-study.

Figure 3: Kaplan–Meier plots displaying 180-day survival stratified according to quartiles of tau fragments at day 1 and day 2. Mortality across quartiles of tau fragments was compared with the log-rank test.

Figure 4: Receiver-operating characteristics curves depicting the performance of tau-A and tau-C in predicting poor neurological outcome defined as a cerebral performance category score of 3-5 180-day after out-of-hospital cardiac arrest at day 1, day 2, day 3. AUC; area under the curve.

Table 1

Demographic variables and comorbidities of the tau fragment study cohort stratified by neurological outcome after 180 days. Good neurological outcome was defined as a cerebral performance category score of 1-2 and poor outcome was defined as 3-5. Data are presented as mean±SD or median and lower to upper quartile (q1-q3) as appropriate. The p-value represents comparison between groups. COPD indicates chronic obstructive pulmonary disease; CPR, cardiopulmonary resuscitation; IHD, ischemic heart disease; ROSC, return of spontaneously circulation; CAG, coronary angiogram; PCI, primary coronary intervention;

	Good neurological outcome, n=103 (62%)	**Poor neurological outcome, n=62 (38%)**	**p-value**
Demography:			
- Age - year (±SD)	59 (±11)	65 (±11)	**0.0003**
- Male sex - n (%)	90 (87%)	55 (89%)	0.79
Randomization:			
- TTM at 36 °C - n (%)	55 (53%)	27 (44%)	0.22
Cardiac arrest characteristics:			
- Witnessed arrest - n (%)	99 (96%)	49 (79%)	**0.0005**
- Bystander CPR - n (%)	84 (82%)	47 (76%)	0.38
- Shockable initial rhythm - n (%)	99 (96%)	47 (76%)	**<0.0001**
- Time to ROSC - min. (IQR)	19 (12–27)	25 (17–37)	**0.001**
- Lactate at admission - min. (IQR)	5 (3-9)	9 (5-14)	**0.003**
- ST-elevations at admission - n (%)	60 (58%)	37 (60%)	0.90
- Acute CAG - n (%)	78 (76%)	51 (82%)	0.33
- PCI - n (%)	53 (51%)	38 (61%)	0.22
Pre-arrest comorbidities:			
- Coronary disease - n (%)	24 (23%)	10 (16%)	0.27
- Previous AMI - n (%)	18 (17%)	7 (11%)	0.28
- Congestive heart failure - n (%)	3 (3%)	3 (5%)	0.42
- Hypertension - n (%)	28 (27%)	22 (35%)	0.26
- Previous TCI/stroke - n (%)	8 (8%)	3 (5%)	0.47
- Diabetes - n (%)	12 (12%)	10 (16%)	0.41
- Asthma/COPD - n (%)	2 (2%)	1 (3%)	0.60
- Alcoholism - n (%)	0 (0)	1 (2%)	0.20
- Comorbidity - score (IQR)	1 (0-2)	1 (0-2)	0.41

Table 2

Tau fragments at admission and after 24, 48 and 72 hours and total Tau after 24, 48 and 72 hours stratified according to target temperature level. Data are presented as median and lower to upper quartile (IQR). The P value represents comparison between groups (TTM33 and TTM36) by Wilcoxon two-sample. TTM33, targeted temperature management at 33°C; and TTM36, targeted temperature management at 36°C.

	TTM33 (n=79)	**TTM36 (n=79)**	**p-value**
tau-A			
- Admission (T0) – ng/L (IQR)	12 (9-20)	13 (9-16)	0.93
- After 24 hours (T24) – ng/L (IQR)	52 (39-65)	58 (45-73)	0.11
- After 48 hours (T48) – ng/L (IQR)	72 (55-83)	72 (59-89)	0.35
- After 72 hours (T72) – ng/L (IQR)	74 (60-89)	78 (69-95)	0.09
tau-C			
- Admission (T0) – ng/L (IQR)	14 (10-20)	13 (10-18)	0.31
- After 24 hours (T24) – ng/L (IQR)	37 (28-49)	38 (31-50)	0.17
- After 48 hours (T48) – ng/L (IQR)	44 (35-57)	51 (40-60)	0.26
- After 72 hours (T72) – ng/L (IQR)	46 (36-57)	50 (40-62)	0.17
Total-tau			
- After 24 hours (T24) – ng/L (IQR)	2 (1-9)	4 (2-14)	0.26
- After 48 hours (T48) – ng/L (IQR)	3 (1-12)	3 (1-13)	0.69
- After 72 hours (T72) – ng/L (IQR)	2 (1-9)	2 (1-11)	0.62

Table 3

Correlations between tau fragments and biomarkers within the first 72 h after out-of-hospital cardiac arrest.

	Tau-A		**Tau-C**		**Total Tau**	
	r^2	p-value	r^2	p-value	r^2	p-value
T0 (n=158)						
- Tau-C (ng/L)	**0.37**	**<0.0001**				
- Total-tau (ng/L)						
T24 (m=149)						
- Tau-C (ng/L)	**0.45**	**<0.0001**				
- Total-tau (ng/L)	0.003	0.49	0.002	0.62		
- CRP (mg/L)	**0.15**	**<0.0001**	**0.26**	**<0.0001**	0.02	0.06
- Leucocytes (10^9/L)	0.008	0.28	0.014	0.15	**0.05**	**0.005**
- Procalcitonin (µg/l)	**0.03**	**0.04**	**0.06**	**0.003**	**0.25**	**<0.0001**
- Hgb (mmol/L)	**0.03**	**0.03**	**0.03**	**0.03**	0.02	0.06
T48 (n=145)						
- Tau-C (ng/L)	**0.49**	**<0.0001**				
- Total-tau (ng/L)	0.003	0.48	0.003	0.55		
- CRP (mg/L)	**0.14**	**<0.0001**	**0.23**	**<0.0001**	0.03	0.06
- Leukocytes (10^9/L)	**0.03**	**0.02**	0.01	0.18	0.01	0.18
- Procalcitonin (µg/l)	0.01	0.19	0.01	0.22	**0.13**	**<0.0001**
- Hgb (mmol/L)	0.02	0.06	**0.04**	**0.01**	0.01	0.19
T72 (n=145)						
- Tau-C (ng/L)	**0.45**	**<0.0001**				
- Total-tau (ng/L)	0.012	0.2	0.004	0.44		
- CRP (mg/L)	**0.08**	**0.0007**	**0.18**	**<0.0001**	0.004	0.46
- Leukocytes (10^9/L)	0.0002	0.87	0.01	0.24	0.01	0.24
- Procalcitonin (µg/l)	0.02	0.09	0.001	0.71	**0.07**	**0.002**
- Hgb (mmol/L)	0.005	0.39	0.006	0.38	0.02	0.14