Title:

Total-tau and neurofilament light in CSF reflect spinal cord ischaemia after endovascular aortic repair

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Running title:

CSF biomarkers in endovascular aortic intervention

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Abstract

BACKGROUND: Repair of extensive aortic disease may be associated with spinal cord ischaemia (SCI). Here we test if levels of cerebrospinal fluid (CSF) biomarkers for neuronal injury are altered in patients with SCI after advanced endovascular repair in extensive aortic disease.

METHODS: CSF was sampled for up to 48 h in ten patients undergoing endovascular aortic repair and analyzed for the axonal damage markers total-tau (T-tau) and neurofilament light (NFL).

RESULTS: Six of ten patients developed SCI (clinically present within 3-6 h). CSF levels of NFL increased up to 37-fold in patients with, but were stable in patients without, SCI. CSF levels of T-tau also increased in patients with SCI, but with some overlap with patients without SCI. Levels of NFL and T-tau did not increase until after the appearance of clinical signs of neurological dysfunction (12-48 h after aortic repair).

CONCLUSIONS: The CSF biomarkers NFL and T-tau both reflect development of SCI after endovascular aortic repair, but do not rise until after clinical signs of SCI appear. Future studies are desirable to further evaluate potential use of these biomarkers for assessment of the severity of SCI, and also to identify earlier biomarkers of SCI.

Keywords: Aortic aneurysm, biomarker, cerebrospinal fluid, endovascular, NFL, tau.
**Introduction**

Modern endovascular techniques are clinically recognized alternatives to open surgery for aortic aneurysm repair. Nevertheless, extensive stent graft coverage of the thoracoabdominal aorta, without revascularization of the spinal arteries, compromises blood flow to the spinal cord and may lead to various degrees of ischaemia (1). The pathophysiology of spinal cord ischaemia is multifactorial (2-4), and several approaches have been proposed to optimize oxygen supply of the spinal cord and prevent this feared complication. Accordingly, maintaining appropriate arterial oxygenation, avoiding low mean arterial pressure levels by infusion of sympathomimetics, avoiding low blood levels of haemoglobin by transfusion of erythrocytes, and avoiding high intrathecal pressure levels by passive drainage of cerebrospinal fluid (CSF), are all recognized and well-established clinical measures for perioperative spinal cord protection in endovascular practice (5-7).

Biomarkers released into the CSF have also been used to identify pathological processes within the central nervous system (8-10). The proteins total tau (T-tau) and neurofilament light (NFL) are both enriched in neurons and released into CSF following axonal or neuronal injury. Increased CSF levels of T-tau and NFL have been reported in patients with acute neurological disease such as ischemic stroke, subarachnoid haemorrhage and traumatic brain injury (8 + Hesse 2000 + Öst 2006 + Nylen 2006) as well as chronic neurodegenerative disease (9), and CSF levels of both biomarkers have also been proposed to reflect therapeutic effects in those patients. In contrast to the biomarker S-100B (10), CSF levels of T-tau or NFL have not been reported in patients subjected to endovascular aortic intervention.

This study was designed to evaluate if perioperative ischaemic spinal cord dysfunction is associated with release of T-tau or NFL into CSF in patients subjected to endovascular repair of the thoracoabdominal aorta.
Patients and methods

Patients
This prospective clinical study was carried out in ten patients (nine men), aged 68 (range 62-77) years and scheduled for endovascular exclusion of thoracoabdominal aortic aneurysm at the Vascular Centre, Skåne University Hospital, Malmö, Sweden, between September 2011 and August 2012.

The study was approved by the Regional Ethical Review Board at Lund University (Dnr 2010/729), and individual informed consents were obtained from all patients.

All patients were at high risk of developing spinal cord ischaemia and assessed by a vascular surgeon to be in need of perioperative drainage of CSF. Nine patients were treated electively and one acutely. No patient had clinical signs of neurological dysfunction at the time of admission for surgery.

Perioperative procedures
The patients were given oral premedication with oxycodone hydrochloride 10 mg, paracetamol 1g and diazepam 5-10 mg approximately 40 min before arrival in the operating room.

An intrathecal 16 G (1.7 mm) Perifix® 431 epidural catheter (B. Braun AG, Melsungen, Germany) was inserted at mid-lumbar (L2-L4) level, and an SW-161-S-03 Hanni-Set (Smiths Medical Deutschland GmbH, Grasbrunn, Germany) for drainage of cerebrospinal fluid was attached as an open system, positioned at approximately 13 cm above the external auditory meatus to enable passive drainage of CSF at a thoracolumbar intrathecal pressure of approximately 10 mm Hg in the flat supine position.
A radial artery was cannulated at wrist level with a 20 G teflon arterial catheter (Becton Dickinson, Helsingborg, Sweden) for blood pressure monitoring and blood sampling.

General anaesthesia was induced intravenously (iv) with 1.5-2.5 mg/kg of propofol or 3-7 mg/kg of thiopental, and maintained with 4-7 % expired concentration of desflurane. Muscle relaxation for oral endotracheal intubation was achieved with 0.6 mg/kg of rocuronium. Supplementary analgesia was provided by target-controlled iv infusion of remifentanil at 3-8 ng/ml.

Individual time points for induction of anaesthesia, start of surgery, endovascular stent graft in position, and end of surgery were recorded.

The lumbar intrathecal pressure was maintained at approximately 10 mm Hg by passive drainage of up to 15 ml/h of CSF until no longer considered to be required, and the intrathecal catheter was maintained for 24-72 hours depending on the extent of postoperative clinical signs of spinal cord dysfunction.

The mean arterial pressure was maintained above 80 mmHg by iv infusions of 0.1-1.8 mg/h of noradrenaline (in all patients but one) and of 5-10 mg/h of dobutamine (in one patient). Blood haemoglobin levels were maintained above 110 g/l by transfusion of erythrocytes.

Clinical follow-up

All patients were admitted for postoperative intensive care, including careful titration and monitoring of arterial blood pressure and thoracolumbar intrathecal pressure levels, and regular clinical assessments of neurological function (level of consciousness, motor function and sensibility), according to local clinical routine procedures.

Cerebrospinal fluid sampling
Samples of CSF were obtained before and after the induction of anesthesia, and 1, 3, 6, 12, 24 and 48 hours after endovascular thoracoabdominal exclusion of the aneurysm, aliquoted in 0.5-millilitre portions, and frozen at -80 °C for later analysis. Some scheduled CSF samples could not be obtained because of temporary catheter dysfunction, catheter removal within 24 hours in the absence of neurological dysfunction, or patient discharge within 48 hours from the intensive care unit.

*Cerebrospinal fluid analyses*

Samples of CSF were shipped frozen to the Clinical Neurochemistry Laboratory in Mölndal, Sweden, for determination of T-tau and NFL levels.

T-tau was analyzed by the INNO-BIA AlzBio3 assay (Innogenetics, Gent, Belgium) on the bead-based xMAP platform (Luminex Inc, Austin, TX, USA), as described elsewhere (11). The lower limit of quantification for this assay was 8.7 ng/l, and the coefficient of variation was 5.2 %.

NFL was measured with a new sensitive sandwich ELISA method (NF-light ELISA kit, Uman Diagnostics AB, Umeå, Sweden) as described by the manufacturer. The lower limit of quantification for this assay was 50 ng/l, and the coefficient of variation was 14 %.

All analyses were made by experienced and board-certified laboratory technicians blinded to individual clinical data.

*Statistical analysis*

Cerebrospinal fluid levels of T-tau and NFL are reported as individual values in figures.

The Mann-Whitney U-test was used to compare baseline and maximum CSF levels of T-tau and of NFL between patients with or without clinical signs of neurological dysfunction.
Results

Demographic and perioperative data

Individual demographic and perioperative data is reported in Table 1.

[TABLE 1 NEAR HERE]

Clinical follow-up

In summary, six patients had postoperative clinical signs of neurological dysfunction within six hours after exclusion of the aneurysm, and half of them had signs of spinal cord injury already within three hours (Fig. 1).

In all six patients with clinical signs of neurological dysfunction, the perioperative period of CSF drainage was extended to 72 hours, and to further optimize arterial perfusion of the spinal cord, the local intrathecal pressure was reduced and maintained below 6 mm Hg, and the mean arterial pressure was raised and maintained above 90 mm Hg. The neurological function partially improved in two patients during this period.

The median duration of primary hospital care was 14 (range 4-57) days. Four patients were discharged to other hospital facilities, one to a rehabilitation facility, and two to a municipal short-stay facility. The remaining three patients returned home.

At the time of discharge, five patients had clinical signs of incomplete, and one of complete, spinal cord dysfunction. Two patients were in need of a wheelchair, and four needed other walking aids.

[FIGURE 1 NEAR HERE]

Cerebrospinal fluid levels of T-tau
There was no significant difference in baseline levels of T-tau between patients with or without postoperative neurological dysfunction \((P >0.30)\). CSF levels of T-tau gradually increased by 2-400\% of baseline values within 12 hours in four, and within 48 hours in the remaining two, patients with neurological complications (Fig. 1-2). In contrast, T-tau levels did not change, or transiently doubled at 6-12 hours, in the remaining four patients with normal neurological function.

**[FIGURE 2 NEAR HERE]**

*Cerebrospinal fluid levels of NFL*

There was no significant difference in baseline levels of NFL between patients with or without postoperative neurological dysfunction \((P >0.300)\). During the study period, NFL levels increased by two to 37 times at 24-48 hours after exclusion of the aneurysm in all six patients with clinical signs of neurological dysfunction (Fig. 1) but not in the remaining ones \((P = 0.006)\). No 48-hour samples were obtained in patients with normal neurological function, but all three patients where 24-hour samples were obtained had lower values of NFL than four of five patients with neurological dysfunction (Fig. 3).

**[FIGURE 3 NEAR HERE]**

**Discussion**

We found that all patients with neurological dysfunction after endovascular repair of thoracoabdominal aortic aneurysm had increased CSF levels of neuronal injury biomarkers within 48 hours, and that CSF levels of NFL increased considerably more than of T-tau. Furthermore, there were early transient increases in T-tau also in some patients with normal postoperative neurological function.
Our finding that all patients with neurological complications had increased CSF levels of NFL, and that no such increase was found in patients without complications at 24 hours, strongly suggest that NFL is released into CSF from neurons damaged by the therapeutic intervention.

Severe spinal cord dysfunction may result from endovascular exclusion of thoracoabdominal aortic aneurysm (1-4), but to our knowledge this is the first study of associations between such complications and CSF biomarkers for neuronal and axonal damage. Increased CSF levels of both tau and NFL have previously been reported in both acute and chronic brain disease or injury. For example, a marked increase in the CSF levels of NFL is found after acute brain insults such as ischaemic stroke, subarachnoid haemorrhage and brain trauma, and correlated with clinical outcome (8, 9 + fler refs enligt intro) and increased levels have also been proposed to reflect the extent of repetitive head trauma in amateur boxers (12).

Individual increases in CSF levels of T-tau and NFL were found to be delayed by twelve hours or more compared with the onset of clinical signs of neurological dysfunction. Whereas neurological signs of spinal cord dysfunction were obvious within 3-6 h of the intervention, neither CSF levels of T-tau nor NFL increased until after 18-48 h. It also seems that T-tau responds earlier – several hours before NFL – to ischaemic spinal cord injury in this clinical context. As a matter of fact, NFL was not detected in CSF until 48 hours after the intervention in patients with neurological dysfunction.

It is also noteworthy that T-tau levels increased later in the patient who developed complete spinal cord ischaemia than in patients with clinical signs of partial ischaemia, but higher CSF levels were found in all patients with neurological complications. However, there were overlaps in both absolute and relative increases in CSF levels of T-tau between patients with or without complications.
The finding that CSF levels of biomarkers of neuronal injury gradually increase in patients with major neurological complications after endovascular interventions for thoracoabdominal aortic aneurysm opens for the possibility that such measurements could be used to earlier identify patients at risk of future severe complications. However, increased levels of the CSF biomarkers studied here, T-tau and NFL, were not found until long after the first clinical signs of neurological dysfunction, which makes them unsuitable for early detection of neuronal injury in this context.

A main limitation of this study is the small sample size, although serial CSF samples were obtained in each patient. Nevertheless, the effect size of the change in CSF levels of NFL was large enough to statistically confirm association with postoperative clinical signs of neurological dysfunction.

Another study limitation is the lack of 48-hour CSF samples in patients without neurological sequelae, excluding later postoperative comparison with neurologically impaired patients with respect to CSF levels of T-tau and NFL.

We conclude that NFL is released into CSF after ischaemic spinal cord injury, since increased CSF levels of NFL were found in patients with neurological complications after endovascular exclusion of thoracoabdominal aortic aneurysm but not in patients without such complications. Future studies should aim at identifying biomarkers reflecting neuronal injury before clinical signs of neurological dysfunction appear.

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**Author contributions**

All authors designed the study. EM managed the patients. NM, HZ and KB were responsible for biochemical analyses. EM, NM and JÅ made statistical analyses, and EM, NM and JÅ drafted the paper. All authors revised the paper for intellectual content. The final version of the paper was approved by all authors.

**References**


Tables
**Figure legends**

Figure 1.

Schematic presentation of clinical neurological signs and increased (>50 % above baseline) cerebrospinal fluid (CSF) levels of the biomarkers total-tau (T-tau) and neurofilament light (NFL) in six patients with neurological dysfunction after endovascular exclusion of thoracoabdominal aortic aneurysm.

Figure 2.

Cerebrospinal fluid (CSF) levels of the biomarker total-tau (T-tau) in patients undergoing endovascular exclusion of thoracoabdominal aortic aneurysm. CSF samples were obtained before, at the time of (stent graft in position), and at 1, 3, 6, 12, and 48 hours after, exclusion of the aneurysm. The left panel shows raw data and the right panel shows data normalized to the first sample. The patients are coded according to whether they developed neurological clinical dysfunction (NC) or not (OK).

Figure 3.

Cerebrospinal fluid (CSF) levels of the biomarker neurofilament light (NFL) in patients undergoing endovascular exclusion of thoracoabdominal aortic aneurysm. CSF samples were obtained before, at the time of (stent graft in position), and at 1, 3, 6, 12, and 48 hours after, exclusion of the aneurysm. The left panel shows raw data and the right panel shows data normalized to the first sample. The patients are coded according to whether they developed neurological clinical dysfunction (NC) or not (OK).
Figure 1.