Aus der Klinik und Poliklinik für Anästhesiologie und Intensivtherapie

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Entzündliche Erkrankungen des zentralen Nervensystems mit intensivmedizinischer Relevanz: Definitionen, Diagnostik, Prognoseabschätzung und Therapieoptionen

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INHALTSVERZEICHNIS

EINLEITUNG

Systemische Inflammation ist die allgemeine Antwort des Organismus auf eine Schädigung, die durch verschiedene Faktoren, wie Traumata, Operationsreize, extrakorporale Therapieverfahren, chemische Noxen oder auch pathogene Erreger ausgelöst werden kann [1,2]. Eine initial systemische Inflammation kann das zentrale Nervensystem (ZNS) sekundär schädigen und zu klassischen enzephalopathischen Syndromen wie dem Delir oder, im Rahmen einer Infektion, zu einer Sepsis-assoziierten Enzephalopathie (SAE) führen [3-5]. Dieser Prozess ist divergent zu primären ZNS-Erkrankungen, die sich im ZNS ausbilden und infektiöser sowie nicht-infektiöser Genese sein können [6-8]. Gemeinsam ist den primären wie auch sekundären ZNS-Erkrankungen, dass neuroaxonale Schädigung von Hirnstrukturen durch neuroinflammatorische sowie neurodegenerative Prozesse auftreten kann [6,8,9].

Ein klassischer Vertreter der immun-mediierten primären ZNS-Erkrankungen stellt die Multiple Sklerose (MS) dar [6,10,11]. Eine typische sekundäre ZNS-Erkrankung ist die SAE, die sich im Zuge einer primär systemischen Infektion erst im weiteren Verlauf im ZNS manifestiert [5,12,13]. Gemeinsam ist der MS und der SAE, dass eine neuroaxonale Schädigung im ZNS eintritt und im Rahmen neurodestruktiver Vorgänge langfristige neurologische Defizite nach sich ziehen kann [6,14,15].

Es soll zunächst auf die primäre, autoimmunologisch vermittelte neuroaxonale Schädigung bei der MS eingegangen werden, bei der der Fokus auf der Unterdrückung der zerebralen Inflammationsreaktion und dem Therapieansprechen im Rahmen des Therapeutischen Plasmaaustausches (TPA) liegen soll. Nachfolgend soll der Nachweis der neuroaxonalen Schädigung bei der sekundären ZNS-

Schädigung im Rahmen der SAE dargestellt werden, um die Diagnostik und Prognoseabschätzung im Rahmen der Sepsis zu ermöglichen.

Multiple Sklerose als klassische, autoimmunologisch vermittelte Erkrankung des ZNS

Bei der MS handelt es sich um eine immun-vermittelte, demyelinisierende Erkrankung des ZNS, von der weltweit mehr als 2,5 Mio. Menschen betroffen sind [16-18]. Das Klinisch isolierte Syndrom (KIS) ist definitionsgemäß eine Erstmanifestation einer potenziellen MS, bei der die gleichen Symptome auftreten können, jedoch die Diagnosekriterien der zeitlichen und räumlichen Dissemination noch nicht komplett erfüllt sind [19,20].

Die Pathophysiologie von KIS und MS wird als immun-mediiert angesehen, wenn auch die exakten Mechanismen noch nicht geklärt werden konnten [6,21,22]. Vermutet wird eine durch genetische Prädisposition sowie umwelt-assoziierte Antigenexposition (z.B. eine virale Infektion) ausgelöste Aktivierung von T-Zellen, die nach peripherer Aktivierung die Blut-Hirn-Schranke passieren können und autoreaktiv einen lokalen inflammatorischen Prozess an der Myelinscheide der Nervenzellen auslösen [23-25]. Dies kann zur Aktivierung von Microglia im ZNS und somit zur Unterhaltung des Inflammationsprozesses führen, auch wenn keine neuen T-Zellen ins ZNS eindringen [23,25]. Neuere Daten zeigen, dass im ZNS ebenfalls eine Antigen-bedingte B-Zellantwort im Rahmen der MS stattfindet, was eine Erklärung für die Antikörper- und B-Zell-depletierenden Therapiemaßnahmen darstellt [26-28].

KIS und MS präsentieren sich in Abhängigkeit der entsprechenden Lokalisation entzündlicher Herde im ZNS sehr variabel und klinisch eindrücklich besonders im Rahmen von Krankheitsschüben [29,30]. Ein Krankheitsschub ist als definitiv neue neurologische Symptomatik definiert, die mindestens 24h anhält und nicht durch Fieber oder einen Infekt bedingt ist [30]. Die klinische Symptomatik kann verschiedene neurologische Funktionssysteme betreffen und typische neurologische Defizite, wie eine Neuritis nervi optici, zerebelläre Symptome wie Ataxie, Sensibilitätsstörungen oder auch Paresen mit Spastik umfassen [11,30,31]. Zur Beurteilung betroffener Funktionssysteme im ZNS sowie zur Festlegung der Krankheitsschwere im Rahmen der MS wird die Expanded Disability Status Scale (EDSS) verwendet [32]. Dabei handelt sich um eine Skala zwischen 0 (keinerlei neurologische Defizite) und 10 (Tod im Rahmen der MS) [32]. Das Risiko für diverse sekundäre Komplikationen im Rahmen der Grunderkrankung sowie im Rahmen der MS-spezifischen Akut- und Langzeittherapie ist bei den Patienten deutlich erhöht [33-35]. Thrombosen, Lungenarterienembolien, anaphylaktische Reaktionen sowie die Entwicklung einer Sepsis im Rahmen der akuten Schubtherapie haben häufig intensivmedizinische Relevanz und Behandlungsbedürftigkeit [34,36,37].

Die Diagnosestellung eines KIS und einer MS basieren auf den aktuell gültigen Diagnosekriterien der MS [38]. Im akuten Krankheitsschub ist mindestens der klinische, besser noch ergänzend der bildmorphologische und ggf. elektrophysiologische Nachweis der Krankheitsaktivität indiziert, bevor Therapiemaßnahmen eingeleitet werden [23,25,39]. Neben den klinischen und bildmorphologischen Befunden sind auch Biomarker zum Nachweis von Neuroinflammation, Krankheitsprogression und neuroaxonaler Schädigung von Relevanz [40-42]. Neurofilamente in Blut und Liquor cerebrospinalis gehören zu den

axonalen Schädigungsmarkern und werden bei der MS zur Diagnostik und Therapiesteuerung verwendet [43-45]. Insbesondere die wiederholte Messung der Neurofilamente im Blut bietet die Möglichkeit eines für den Patienten komfortablen und wenig belastenden Monitorings der Krankheitsaktivität und kann die Notwendigkeit aufwändiger Bildgebungsverfahren, wie eines zerebralen MRT, reduzieren helfen [43].

Im Rahmen eines akuten MS-Schubes sollte die akute zerebrale Inflammationsreaktion möglichst frühzeitig unterdrückt werden, um eine Symptombesserung zu erzielen [35,39]. Es wird eine Stufeneskalationstherapie im Rahmen eines akuten Krankheitsschubes empfohlen [39,46,47]. Die Eskalationstherapie umfasst zunächst die hochdosierte Gabe von Glukokortikosteroiden (GKS), deren insgesamt breite immunsuppressive Wirkung spezifisch durch eine Apoptose peripherer Leukozyten erklärt wird, was die Neuroinflammation durch Downregulation der T-Zell-Aktivität unterdrücken soll [48]. Bei Versagen der GKS-Therapie oder vorliegenden Kontraindikationen ist der Therapeutische Plasmaaustausch (TPA) als nächste Stufe der Eskalationstherapie empfohlen [47,49,50]. Dessen Wirkung wird durch eine Elimination von im Plasma zirkulierenden humoralen Faktoren, wie Antikörpern, Komplementfaktoren, Zytokinen und Immunkomplexen erklärt, die am Prozess der Neuroinflammation und neuroaxonalen Schädigung durch Demyelinisierung im ZNS beteiligt sind [51-53]. Durch den TPA können bei 40-90% der primär GKS-nicht-responsiven Patienten noch Verbesserungen der neurologischen Defizite erreicht werden [54-56].

Das neurologische Outcome der MS-Patienten ist inter-individuell sehr variabel und nicht sicher vorhersagbar [29,57,58]. Trotz aller therapeutischen Bemühungen ist

über den Langzeitverlauf der MS dennoch leider weiterhin mit einer zunehmende Rate an hilfs- und pflegebedürftigen Patienten zu rechnen [59].

Sepsis-assoziierte Enzephalopathie (SAE)

Die Sepsis hat ungeachtet aller Entwicklungen in Diagnostik und Therapie eine hohe Inzidenz und Letalität [60,61]. Die therapeutischen Bemühungen bezogen sich in den letzten Dekaden im Wesentlichen auf das kurz- und langfristigen Überleben der Patienten, wobei die Überlebensrate von Patienten mit Sepsis durch verbesserte Therapiemaßnahmen erhöht werden konnte [61,62]. Wesentlichen Einfluss auf die Morbidität und Letalität sowie auf das langfristige neurokognitive Outcome dieser Patienten hat die Sepsis-assoziierte Enzephalopathie (SAE) [15,63]. Bei der SAE handelt es sich um eine diffuse Störung der Hirnfunktion, die im Rahmen der Sepsis auftritt, nicht jedoch durch eine primäre ZNS-Infektion oder andere Ursachen der Enzephalopathie verursacht wird [12].

Die Pathophysiologie ist trotz intensiver Forschungsbemühungen bisher nicht vollständig verstanden [64-66]. Tierexperimentelle Arbeiten zeigten diverse potentielle Mechanismen der SAE, wobei die Pathogenese wahrscheinlich multifaktoriell bedingt ist [67-70]. Relevant zu sein scheinen insbesondere die Microglia-Aktivierung, mitochondriale Dysfunktion, oxidativer Stress, die Störung der Blut-Hirn-Schranke und die Neuroinflammation, die über eine neuroaxonale Schädigung zu einer Neurodegeneration führen können [67,69,71]. Humane neuropathologische Arbeiten legen nahe, dass spezifische Hirnregionen, insbesondere die Frontalhirnregion, der Hippocampus, die Amygdala und der Hirnstamm im Rahmen der SAE geschädigt werden (Abbildung 1) [14]. In

Zusammenschau aller pathophysiologischer Hypothesen zur SAE wurden in den letzten Jahren die neuroinflammatorische und vaskuläre Hypothese hervorgehoben [13,14]. Bei der neuroinflammatorischen Hypothese wird im Rahmen der Sepsis von einer peripheren Aktivierung von Gewebemakrophagen ausgegangen, die zu einer Freisetzung von Entzündungsmediatoren (u.a. IL-1β, IL-6, TNFα) aus dem Gefäßendothel von Hirngefäßen führen. Dies führt zu einer primären Aktivierung der Microglia-Zellen, deren sezernierte Entzündungsmediatoren zur axonalen Dysfunktion und Schädigung bis hin zur Degeneration führen können (Abbildung 1) [14]. Die vaskuläre Hypothese besagt, dass es im Rahmen der Sepsis zu Hirnischämien sowie Mikro- und Makrohämorrhagien kommen kann, die ebenfalls zur zerebralen Schädigung führen (Abbildung 1) [14,72].

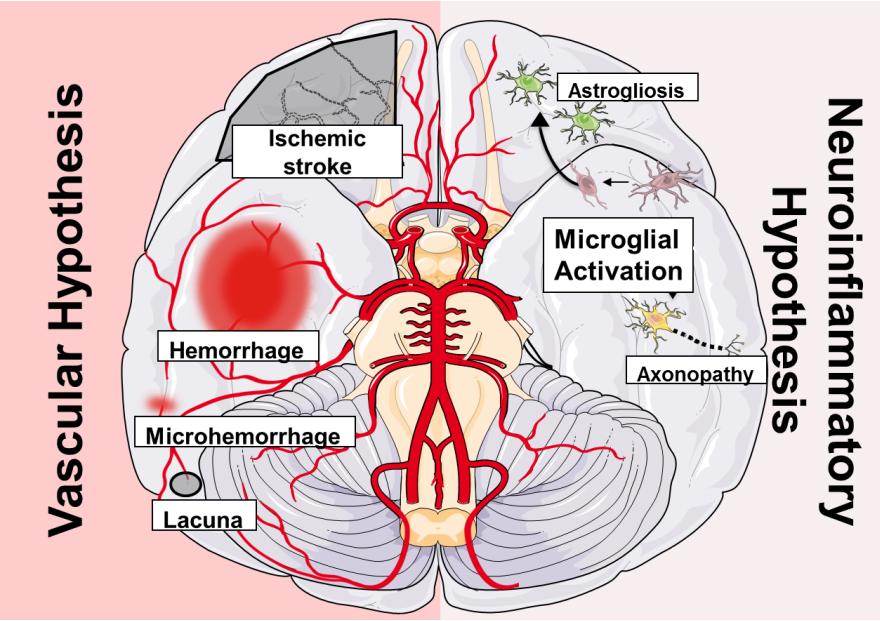

Abbildung 1: Hypothesen zur Pathogenese der Sepsis-assoziierten Enzephalopathie.
Mit freundlicher Genehmigung von Prof. Tarek Sharshar, Institut Pasteur, Paris.

Die SAE ist im Vergleich zu anderen Sepsis-induzierten Organdysfunktionen eine typischerweise sehr frühzeitig einsetzende Komplikation [5]. Plötzlich auftretende Änderungen des mentalen Status im Sinne von Verwirrtheit, Agitation,

Desorientierung, sowie Somnolenz bis hin zu komatösen Zuständen zählen zum klinischen Bild des Patienten mit SAE [13,73]. Der Schweregrad der Enzephalopathie korreliert mit der Letalität der Patienten [73]. Patienten mit SAE zeigen teilweise selbst Jahre nach überlebter Sepsis noch neurokognitive Defizite, was hohe sozioökonomische Relevanz hat [15,63,74].

Insbesondere bei Patienten mit septischem Schock, notwendiger Analgosedierung sowie mechanischer Beatmung ist der rein klinische Nachweis der SAE und deren neuropsychiatrischen Symptome häufig nur eingeschränkt möglich [75,76]. Der Goldstandard zur Diagnosestellung der SAE bzw. eines Delirs ist die fachärztliche neuropsychiatrische Untersuchung unter Verwendung des *Diagnostic and Statistical Manual of Mental Disorders (DSM)* [77]. In der täglichen Routine kommen aber auch primär für das Screening und Monitoring eines Delirs entwickelte Instrumente, wie die Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) oder die Intensive Care Delirium Screening Checklist (ICDSC) zur Anwendung [78-80]. Aufgrund der eingeschränkten Möglichkeiten zur klinischen Evaluation der Patienten sind häufig weitere apparative diagnostische Methoden notwendig [75-77]. Bildgebende Verfahren, hier insbesondere die cMRT, konnten strukturelle Hirnläsionen im Rahmen der Sepsis und SAE nachweisen, die eine Erklärung für die langfristigen neurokognitiven Defizite der Patienten darstellen könnten [81-83]. Des Weiteren spielt die Elektroenzephalographie (EEG) eine Rolle zur Einschätzung des Schweregrades der SAE sowie zu deren Prognoseabschätzung [84-86]. Zusätzlich können wichtige Differentialdiagnosen, wie der non-konvulsive Status epilepticus, mittels EEG ausgeschlossen werden [87]. Die Nahinfrarotspektroskopie (Near Infrared Spectroscopy, NIRS) kann Zusatzinformationen zur zerebralen Oxygenierung, die transkranielle Dopplersonograpie (TCD) der intrakraniellen

Hirnarterien zur zerebrale Durchblutung bei Patienten mit SAE erbringen [76]. Aufgrund des Aufwandes einzelner apparativer Verfahren wird in Abhängigkeit der Erkrankungsschwere von Sepsispatienten häufig in einer Nutzen-Risiko-Abwägung auf die Durchführung einer cMRT verzichtet, die jedoch eine neuroaxonale Schädigung nachweisen könnte und zum Ausschluss anderer Differentialdiagnosen, wie einem Schlaganfall oder einer Hirnblutung, wichtig ist [87,88]. Daher werden Biomarker zum Nachweis einer neuroaxonalen Schädigung bei Sepsis und SAE intensiv untersucht, da deren Abnahme am Patienten unkompliziert ist [89,90]. Je nach Schädigungsort im ZNS werden mehrere Gruppen von Biomarkern unterschieden. Hierzu zählen: 1) Neuronale Schädigungsmarker, unter anderem die Neuronen-spezifische Enolase (NSE), 2) Mikrogliale bzw. astrozytäre Marker, zu denen das S100B-Protein und das Glial Fibrillary Acidic Protein (GFAP) gehören und 3) Axonale Schädigungsmarker, denen die Neurofilamente zugerechnet werden [89,91,92]. Die bei SAE bisher am häufigsten untersuchten Biomarker sind die NSE und das S100B-Protein, die sich aufgrund heterogener Studienergebnisse bisher jedoch nicht als Biomarker in der täglichen Routine durchsetzen konnten [93-95]. Ein Nachteil dieser beiden Biomarker ist die unzureichende axonale Spezifität [89,96]. Neurofilamente sind spezifischer für das axonale Kompartiment der Nerven und wurden bisher bei verschiedenen neurologischen Erkrankungen mit ZNS-Beteiligung untersucht [9,44,97]. Neurofilamente sind als Intermediärfilamente Typ IV Bestandteil des axonalen Zytoskeletts und für die axonale Stabilität essentiell [98]. Nach Länge und Molekulargewicht werden leichte (Neurofilament light chains, NfL), mittelschwere (Neurofilament medium chains, NfM) und schwere Ketten (Neurofilament heavy chains, NfH) unterschieden (Abbildung 2) [98]. Bisher existiert kein valider Biomarker, der in der täglichen Praxis bei SAE eingesetzt wird.

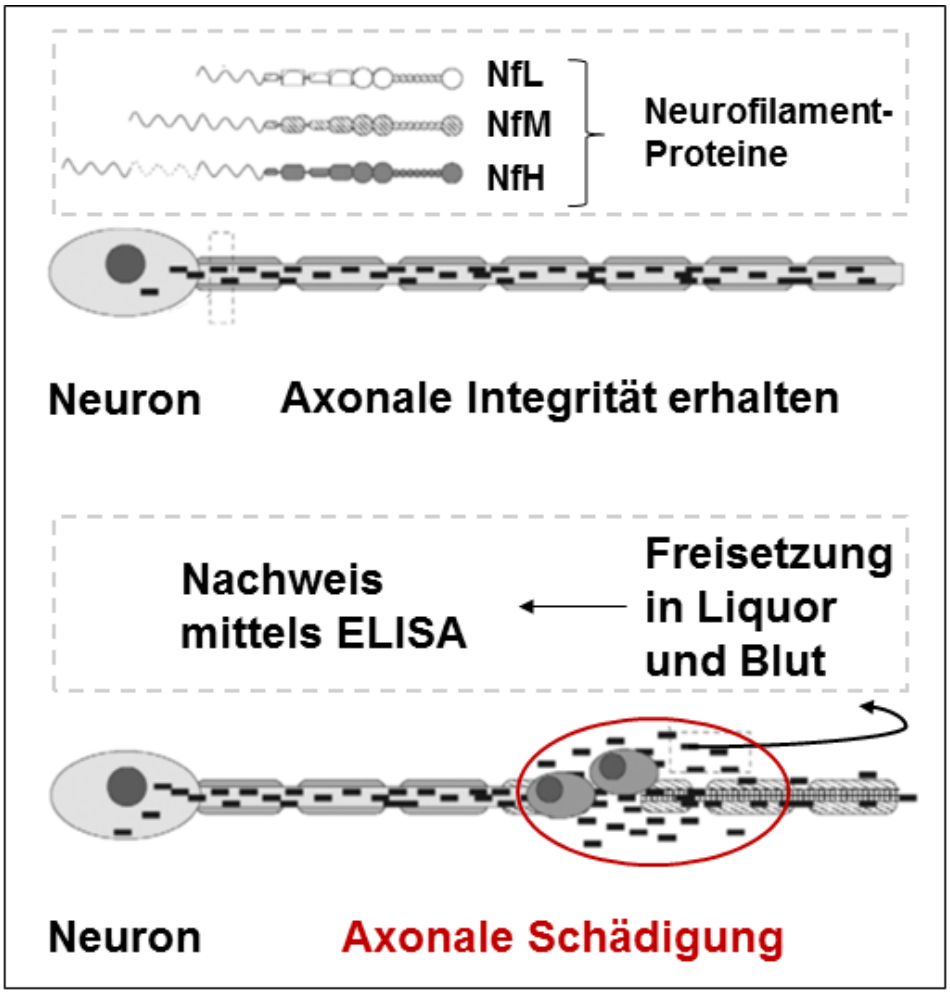

Abbildung 2: Neurofilament-Proteine zur Darstellung der neuroaxonalen Schädigung des Nerven. *Oben:* Axon ohne Schädigung bei intaktem Zytoskelett. *Unten:* Axonale Schädigung mit Freisetzung der Neurofilamente in Blut und/oder Liquor cerebrospinalis, Nachweis mittels spezifischer ELISA-Tests. ELISA, Enzyme-linked immunosorbent assay; NfH, Neurofilament heavy chains; NfL, Neurofilament light chains; NfM, Neurofilament medium chains. Modifiziert nach [98].

Eine selektive, kurative Therapie der SAE ist bisher nicht möglich [12,65,99]. Die frühzeitige und leitliniengerechte Therapie der zu Grunde liegenden Sepsis stellt die Therapie der Wahl dar [13,77,100]. Die fokussierte Vermeidung oder frühzeitige Therapie einzelner Risikofaktoren, die zur Entwicklung einer SAE beitragen, könnte

helfen die SAE sinnvoll zu beeinflussen [65,66,101]. Darüber hinaus kann die SAE bisher, analog zum Delir, nur symptomatisch behandelt werden [12,13]. Immunsuppressive Therapieversuche mit GKS oder TPA im Rahmen der Sepsis zeigten bisher keinen überzeugenden Überlebensvorteil für die Patienten [102-104].

FRAGESTELLUNG, METHODIK UND ERGEBNISSE DER ORIGINALARBEITEN (OA)

Für Patienten mit lebensbedrohlichen, intensivpflichtigen Erkrankungen ist nicht nur das Überleben entscheidend, sondern auch die zu erwartende Lebensqualität [63]. Eine neuroaxonale Schädigung im Rahmen von kritischen Erkrankungen stellt daher eine entscheidende Komplikation dar. Neben körperlichen Einschränkungen mit verminderter Teilnahmefähigkeit am täglichen Leben, sind neurokognitive Defizite (u.a. Merkfähigkeitsstörungen oder Depressionen) ein vergleichbar gravierender Faktor für die Reduktion der Lebensqualität. Mit zunehmender Erkrankungsschwere des Intensivpatienten ist die diagnostische Detektion und Verlaufskontrolle der zerebralen Schädigung erschwert und apparativ aufwendig. Zusätzlich ist nicht jedes diagnostische Verfahren ubiquitär verfügbar.

Das Ziel dieser Arbeit ist es darzustellen, wie die neuroaxonale Schädigung bei typischen Erkrankungen, bei denen Neuroinflammation und Neurodegeneration eine Rolle spielen, nachgewiesen und therapiert werden kann. Dabei soll der Fokus auf dem klinischen, bildmorphologischen und Biomarker-gestützten Nachweis liegen und die Besonderheiten der Anwendung verschiedener Nachweisverfahren bei MS und SAE darstellen. Im Bereich der MS, einer klassisch autoimmunologisch vermittelten primären ZNS-Erkrankung soll auf etablierte diagnostische Nachweisverfahren der zerebralen Entzündungsaktivität sowie auf therapeutische Verfahren zu deren Unterdrückung eingegangen werden (**OA 1 und 2**). Auf dieser Grundlage sollen die im Vergleich zur MS häufig erschwerten Nachweismöglichkeiten der SAE bei kritisch kranken Intensivpatienten präsentiert und Implikationen für die zukünftige Diagnostik und Therapie bei SAE abgeleitet werden (**OA 3-5**). Dazu werden bei der SAE

zunächst tierexperimentelle und humane Studiendaten in einem translationalen Forschungsansatz zum Nachweis potenzieller Pathomechanismen herangezogen (**OA 3**). Die hier nachgewiesene zerebrale Schädigung soll Ausgangspunkt für longitudinale Biomarkerdiagnostik und bildmorphologische Nachweisverfahren im Rahmen der Sepsis und SAE sein (**OA 4 und 5**). Alle Fragestellungen wurden jeweils nach Genehmigung der Tierversuche sowie nach positivem Ethikvotum durchgeführt.

OA 1) Therapeutischer Plasmaaustausch bei Glukokortikosteroid-nicht-responsiven Patienten mit Klinisch Isoliertem Syndrom

Ehler J, Koball S, Sauer M, Hickstein H, Mitzner S, Benecke R, Zettl UK. Therapeutic Plasma Exchange in Glucocorticosteroid-Unresponsive Patients With Clinically Isolated Syndrome. Ther Apher Dial 2014;18:489-96.

Hintergrund: Das KIS repräsentiert als erstes klinisches Ereignis Krankheitscharakteristika einer möglichen MS, ohne dass die diagnostischen Kriterien der MS bereits erfüllt sind.

Fragestellung: Die Datenlage zum TPA bei KIS-Patienten ist, im Gegensatz zur Therapie des akuten MS-Schubes, unbefriedigend. Wir untersuchten daher GKS-nicht-responsive KIS-Patienten und deren Ansprechen auf den TPA.

Material und Methoden: Es wurden retrospektiv GKS-nicht-responsive KIS-Patienten eingeschlossen, die einen TPA erhalten hatten. Diese Patienten erhielten eine klinisch-neurologische Untersuchung unter Erhebung des EDSS, elektrophysiologische Untersuchungen, eine zerebrale MRT- sowie Liquordiagnostik. Primärer Endpunkt für den Therapieerfolg des TPA war die Verbesserung des

führenden klinischen Symptoms/neurologischen Defizits, welches zum TPA geführt hatte. Therapieerfolg wurde unterteilt in a) Deutliche Besserung und b) moderate Verbesserung des Symptoms. Kein Ansprechen auf den TPA wurde als c) keine Verbesserung des Symptoms sowie d) Verschlechterung des Symptoms oder neue Symptome definiert. Als sekundärer Endpunkt kam die Entwicklung der EDSS-Werte unter TPA zur Anwendung. Ein Therapieerfolg des TPA lag bei einer EDSS-Verbesserung um ≥ 1,0 Punkte (bei EDSS < 5,5 vor TPA) und bei einer EDSS-Verbesserung um > 0,5 Punkte (bei EDSS ≥ 6,0 Punkte vor TPA) vor. Der TPA erfolgte leitliniengerecht nach schriftlicher Einwilligung aller Patienten. Es wurden mindestens 3 TPA-Einzelbehandlungen (jeweils Austausch des 1-fachen Plasmavolumens pro Patient) in einem Intervall von 2 Tagen durchgeführt. Das entzogene Plasma wurde durch ein Gemisch aus Ringer-Laktat- und 20%iger Humanalbumin-Lösung ersetzt.

Statistische Berechnungen erfolgten mittels SPSS (IBM SPSS Statistics, Version 20, Chicago, IL, USA). Der Vergleich der EDSS-Werte vor, während und nach TPA erfolgte mittels Wilcoxon-Test. Das Signifikanzniveau lag bei $p<0,05$.

Ergebnisse: Untersucht wurden elf GKS-nicht-responsive Patienten mit KIS und TPA zwischen den Jahren 2001 und 2011 an der Universitätsmedizin Rostock. Das mediane Alter lag bei 28 (20-41) Jahren. Alle Patienten wiesen in der zerebralen MRT-Untersuchung Läsionen im Bereich der weißen Substanz auf, fünf dieser Patienten auch Kontrastmittel-aufnehmende Läsionen als Hinweis auf eine akute zerebrale Schädigung mit Störung der Blut-Hirnschranke. Der TPA erbrachte bei 10 von 11 Patienten eine Symptomverbesserung, die sich als deutliche Verbesserung bei 2 von 11 und als moderate Verbesserung bei 8 von 11 Patienten zeigte. Einer von elf Patienten zeigte keine Verbesserung durch den TPA. Der EDSS-Verlauf vor

TPA (medianer EDSS 3,0 (1,5-7,5 Punkte)) und nach TPA (medianer EDSS 2,0 (1,0-6,0 Punkte)) zeigte eine signifikante Verbesserung unter Therapie (p=0,004).

Die Verlaufskontrolle des neurologischen Status der Patienten, im Median 71,5 (1-187) Tage nach letztem TPA, zeigte bei 10 von 11 Patienten einen medianen EDSS von 2,0 (1,0-6,0) Punkten und somit eine stabile klinische Situation. Lediglich ein Patient zeigte eine Verschlechterung mit Erreichen des Ausgangs-EDSS-Wertes vor TPA. Über den Langzeitverlauf entwickelten 8 von 10 Patienten, die für ein Follow-up zugänglich gewesen waren, eine RR (Relapsing-remitting)-MS, ein Patient eine NMO (Neuromyelitis optica) und ein Patient keine weitere Krankheitsaktivität.

Schlussfolgerung: Bei einer ersten Krankheitsmanifestation im Sinne eines KIS zeigt der TPA bei GKS-nicht-responsiven Patienten eine hohe Ansprechrate und kann die klinisch-neurologischen Symptome im Sinne einer Rekonvaleszenz positiv beeinflussen.

OA 2) Therapeutischer Plasmaaustausch als Rescue-Verfahren bei Patienten mit Klinisch Isoliertem Syndrom und akuter Verschlechterung der Multiplen Sklerose – Eine retrospektive Analyse von 90 Patienten

Ehler J, Koball S, Sauer M, Mitzner S, Hickstein H, Benecke R, Zettl UK. Response to Therapeutic Plasma Exchange as a Rescue Treatment in Clinically Isolated Syndromes and Acute Worsening of Multiple Sclerosis: A Retrospective Analysis of 90 Patients. PLoS ONE 2015;10(8):e0134583.

Hintergrund: Bisher existieren wenige Daten zu TPA bei KIS- und MS-Patienten. **Fragestellung:** Ziel dieser Arbeit war es an einem, im Vergleich zu publizierten Daten, großen Patientenkollektiv, die TPA-Wirkung und Einflussfaktoren auf deren Behandlungserfolg zu untersuchen. Zusätzlich wurden intensivmedizinisch relevante Komplikationen im Rahmen der Schubtherapie analysiert.

Material und Methoden: Es wurden retrospektiv 90 Patienten mit KIS und MS laut den McDonald-Kriterien von 2001 eingeschlossen. Alle Patienten waren im Rahmen der Behandlung ihres akuten Krankheitsschubes GKS-nicht-responsiv und erhielten in ihrem Krankheitsverlauf erstmalig einen TPA. Es wurden sowohl schubförmige Verlaufsformen (KIS, RR-MS) als auch chronische Verlaufsformen, wie die Sekundär-chronisch progrediente (SP)-MS und die Primär-chronisch progrediente (PP)-MS) mit aufgesetzten Schüben eingeschlossen.

Primärer Endpunkt zur Beurteilung der TPA-Wirkung war die klinische Besserung des zum TPA führenden Hauptsymptoms. Die Beurteilung des Therapieerfolges erfolgte analog zu OA 1. Die multimodale Diagnostik der Patienten umfasste eine klinisch-neurologische Untersuchung mit Erhebung des EDSS-Wertes jeweils vor, während und nach TPA sowie elektrophysiologische Diagnostik und zerebrale MRT-Untersuchungen. Der TPA erfolgte standardisiert laut Empfehlungen (siehe OA 1).

Statistische Analysen erfolgten mittels SPSS (IBM SPSS Statistics, Version 20, Chicago, IL, USA). Der univariate Vergleich von Patientencharakteristika in Bezug auf den TPA-Respons erfolgte mittels Chi-Quadrat-Test. Signifikante Parameterunterschiede wurden nachfolgend mittels einer multiplen Regressionsanalyse analysiert. Der Wilcoxon-Test wurde zum Mittelwertvergleich der EDSS-Werte vor, während und nach TPA genutzt. **Ergebnisse:** Es wurden 90 Patienten der Jahre 2001 bis 2013 analysiert (n=21 KIS, n=46 RR-MS, n=18 SP-MS, n=5 PP-MS). Laut primärem Endpunkt zeigten insgesamt 65 von 90 Patienten

(72,2%) eine TPA-Wirkung. Eine deutliche Besserung lag dabei bei 18/90 Patienten (20%) und eine moderate Besserung bei 47/90 Patienten (52,2%) vor. Keine Verbesserung zeigten 25/90 Patienten (27,8%), eine Verschlechterung zeigte kein Patient. Ein Therapieerfolg mittels TPA war signifikant häufiger bei frühen und schubförmigen Verlaufsformen als bei chronischen Verlaufsformen zu registrieren (TPA-Respons KIS vs. SP-MS, p=0,01; RR-MS vs. SP-MS, p=0,002). Der EDSS-Wert aller 90 Patienten vor TPA zeigte eine signifikante Reduktion von 3,75 (1,0-8,5) auf 3,0 (0-8,5) nach TPA (p=0,001). Komplikationen im Rahmen des TPA traten bei 23/90 Patienten (25,6%) auf, waren jedoch nicht signifikant häufiger bei chronischen Verlaufsformen (SP-MS, PP-MS) als bei schubförmigen Verlaufsformen (KIS, RR-MS, p>0,05). Von intensivmedizinischer Bedeutung waren insbesondere eine Katheter-assoziierte Sepsis und eine Lungenarterienembolie im Rahmen des TPA. Die multiple Regressionsanalyse zeigte von diversen untersuchten Faktoren nur die MR-morphologisch vor TPA gesehenen Kontrastmittel-aufnehmenden Herde im Bereich der weißen Substanz als prädiktiven Faktor für den späteren TPA-Respons.

Schlussfolgerung: Mit Hilfe dieser Arbeit konnte eine hohe Ansprechrate bei GKS-nicht-responsiven Patienten mit KIS und MS gezeigt werden. Interessant war dabei, dass frühe und schubförmige Verlaufsformen sowie Patienten mit aktiven, kontrastmittel-aufnehmenden Entzündungsherden im MRT signifikant besser auf den TPA ansprachen. Der TPA sollte daher frühzeitig bei Nicht-Ansprechen auf GKS Anwendung finden, um den aktiven Entzündungsprozess schnellst möglich zu unterdrücken und die Rekonvaleszenz zu ermöglich.

OA 3) Translationaler Nachweis von zwei spezifischen Mechanismen der neuroaxonalen Schädigung im Rahmen der Sepsis – eine longitudinale, prospektive und translationale Studie

Ehler J, Barrett LK, Taylor V, Groves M, Scaravilli F, Wittstock M, Kolbaske S, Grossmann A, Henschel J, Gloger M, Sharshar T, Chretien F, Gray F, Nöldge-Schomburg G, Singer M, Sauer M, Petzold A. Translational evidence for two distinct patterns of neuroaxonal injury in sepsis: a longitudinal, prospective translational study. Crit Care 2017 Oct 23;21(1):262.

Hintergrund: Die Hömöostase der Hirnfunktion verändert sich im Rahmen der Sepsis, wobei es zur Ausbildung einer Sepsis-assoziierten Enzephalopathie (SAE) kommen kann. Die exakten pathophysiologischen Mechanismen der SAE sind bis heute unverstanden.

Fragestellung: Im Rahmen dieser translationalen Arbeit sollten mögliche Mechanismen der neuroaxonalen Schädigung im Tiermodell der Sepsis, postmortal bei verstorbenen Sepsispatienten sowie in vivo bei Patienten mit septischem Schock analysiert werden.

Material und Methoden: Das Studienkonzept dieser longitudinalen, prospektiven und translationalen Studie beinhaltete 1) histologische und immunhistochemische Untersuchungen des Hirngewebes von Ratten mit experimentell induzierter Sepsis durch fäkale Peritonitis, 2) neuropathologische Untersuchungen des Hirngewebes verstorbener Sepsispatienten und 3) eine multimodale in-vivo-Diagnostik zur Detektion der neuroaxonalen Schädigung bei Sepsispatienten unter Nutzung klinischer Delir-, zerebraler MRT-, EEG- sowie longitudinaler Biomarker-Diagnostik.

1. Ratten-Modell der Sepsis: Er erfolgte die Induktion einer Sepsis durch Injektion eines Fäkalgemischs bei adulten Wistar-Ratten (fäkale Peritonitis), die mittels Arterienkatheter und zentralem Venenzugang ein hämodynamisches Monitoring sowie standardisierte Flüssigkeitstherapie erhielten. Das Versuchsprotokoll sah 3 Gruppen vor: a) Ratten ohne Instrumentierung und ohne Sepsis (naive Gruppe, n=4), b) Ratten mit Instrumentierung ohne Sepsis (Sham-Gruppe, n=11) und c) Ratten mit Instrumentierung und Sepsis (Sepsis-Gruppe, n=14). Alle septischen Ratten zeigten nach >12h typische Krankheitszeichen. Die Ratten aller 3 Gruppen wurden unter tiefer Isofluran-Narkose nach 24h, 48h und 72h getötet und das Hirngewebe untersucht.

2. Postmortale Untersuchungen humanen Hirngewebes: Es wurde Hirngewebe von verstorbenen Patienten mit Sepsis (n=5) sowie von verstorbenen Patienten ohne Sepsis (n=3) untersucht. Genau wie bei den Rattenversuchen erfolgte eine standardisierte histologische und immunhistochemische Untersuchung des menschlichen Hirngewebes der Frontal- und Kleinhirnregion mittels H & E-Färbung sowie immunhistochemisch zur Darstellung der Neurofilamente, des GFAP, des ß-Tubulin sowie des ß-Amyloid-Precursor-Proteins (ß-APP).

3. In-vivo-Diagnostik bei Patienten mit Sepsis: Eingeschlossen wurden mindestens 18 Jahre alte Patienten mit schwerer Sepsis und septischem Schock, die keine vorbekannte zerebrale Schädigung aufwiesen. Es erfolgte eine standardisierte multimodale Diagnostik zur Detektion der SAE inklusive Delir-Screening, neuropsychiatrischer Untersuchung, EEG und einer zerebralen MRT zur Darstellung septischer Läsionen der weißen Substanz.

Statistische Untersuchungen erfolgten unter Verwendung der Software SAS (Version 9.4, SAS Institute, Inc., Cary, NC, USA). Der Wilcoxon-Test wurde zum Vergleich nicht-parametrischer Daten genutzt. Beim Vergleich von > 2 unabhängigen Variablen

kam der ANOVA-Test und zur Korrelationsanalyse der Spearmen-Test zur Anwendung. Das Signifikanzniveau lag bei $p<0,05$.

Ergebnisse: *1. Ratten-Modell der Sepsis:* Im Gegensatz zur Gruppe der naiven sowie Sham-Ratten, zeigte sich in der Sepsis-Gruppe eine Korrelation von Neurofilament-Leveln mit der Sepsisdauer. Es wurden 2 Mechanismen der neuroaxonalen Schädigung bei septischen Ratten, nicht jedoch bei den naiven sowie Sham-Tieren gesehen: a) Eine diffuse axonale Schädigung im Bereich der weißen Substanz, die sich mittels ß-APP- und ß-Tubulin-Färbung darstellen ließ und b) Ischämische Läsionen im Bereich der weißen Substanz, die sich mittels H & E- sowie mittels ß-APP-Färbung nachweisen ließen.

2. Humane postmortale Neuropathologie: Identisch zum Tiermodell konnten bei den humanen Hirnschnitten von verstorbenen Sepsispatienten, nicht jedoch bei den nicht-septischen Verstorbenen, die beiden Mechanismen der diffusen axonalen und ischämischen Schädigung nachgewiesen werden.

3. In-vivo-Diagnostik bei Sepsispatienten: Bei 10 von 13 Patienten konnten klinische Symptome einer SAE nachgewiesen werden, bei 3 Patienten gelang dies aufgrund einer Sepsisentwicklung unter Operation und längerfristiger Sedierung nicht. Die EEG-Diagnostik zeigte bei allen 13 Patienten eine teils ausgeprägte Allgemeinveränderung. Auffällig war in der MRT-Diagnostik, dass 9 von 13 Patienten Sepsis-typische Läsionen im Bereich der weißen Substanz als Hinweis auf eine axonale Schädigung aufwiesen. Zusätzlich konnten bei 3 Patienten frische oder subakute Hirnschämien nachgewiesen werden.

Schlussfolgerung: Mittels dieser translationalen Forschungsarbeit konnten diffuse axonale sowie klar umschriebene, lokale ischämische Schädigungen als zwei relevante Mechanismen der neuroaxonalen Schädigung bei Sepsis nachgewiesen

werden. Weitere Untersuchungen zur genauen zeitlichen Entwicklung der Hirnschädigung sollten unter Nutzung von spezifischen Biomarkern folgen.

OA 4) Diagnostische Wertigkeit von NT-proCNP im Vergleich zu NSE und S100B in Liquor cerebrospinalis und Plasma von Patienten mit Sepsis-assoziierter Enzephalopathie

Ehler J, Saller T, Wittstock M, Rommer PS, Chappell D, Zwissler B, Grossmann A, Richter G, Reuter DA, Nöldge-Schomburg G, Sauer M. Diagnostic value of NT-proCNP compared to NSE and S100B in cerebrospinal fluid and plasma of patients with sepsis-associated encephalopathy. Neurosci Lett 2018;692:167-173.

Fragestellung: Bisherige Untersuchungen zur Wertigkeit von Biomarkern bei SAE, hier hauptsächlich der NSE und des S100B zeigten sehr heterogene, teils konträre Ergebnisse und konnten sich in der täglichen Praxis bei SAE bisher nicht etablieren. Für natriuretische Peptide, hier insbesondere das im ZNS am höchsten konzentrierte C-Typ natriuretische Peptid (CNP) und dessen Propeptid, das N-terminale pro-C-Typ natriuretische Peptid (NT-proCNP), konnten verschiedene funktionelle Rollen im ZNS nachgewiesen werden. Im Bereich der endothelialen Glykokalyx triggern inflammatorische Mediatoren wie IL-1ß und TNFα die Freisetzung von NT-proCNP, was zu Veränderungen der Permeabilität führen kann. Weitere Funktionen wurden auch mit Gedächtnisstörungen und synpatischer Plastizität in Zusammenhang gebracht. So führte die CNP-Gabe im Rattenmodell zu veränderter Aktivität im Hippocampus, was zu Gedächtnis- und Lernprozessstörungen führte. Bisherige Daten legen somit eine Rolle von CNP und NT-proCNP bei zerebralen Prozessen

nahe. Eine Untersuchung des NT-proCNP als potenzieller Biomarker bei SAE wurde bisher nicht durchgeführt und stellte somit die Idee dieser Studie dar.

Material und Methoden: Im Sinne einer Proof-of-Concept-Studie wurden Plasma- und Liquorproben von Patienten mit septischem Schock analysiert und mit den Plasma- und Liquorproben neurologischer Patienten verglichen, die keine Hinweise für eine neuroaxonale Schädigung (klinisch, labor-, liquordiagnostisch sowie MR-morphologisch) aufwiesen. Sowohl die Sepsispatienten als auch die neurologischen Kontrollpatienten erhielten standardisiert eine neuropsychiatrische Untersuchung, eine zerebrale MRT-Bildgebung sowie Liquor- und Blutuntersuchungen zur Messung der Biomarker NSE, S100B sowie NT-proCNP. Bei Sepsispatienten erfolgte zusätzlich eine Messung der drei Biomarker über den Zeitverlauf an Tag 1, 3 und 7 im Serum. Ebenfalls wurde bei Sepsispatienten an allen drei Zeitpunkten das Inflammationsausmaß mittels IL-6- und PCT-Messung im Serum bestimmt. Das Outcome in Hinblick auf Tod und funktionelle Eigenständigkeit (Barthel-Index) 100 Tage nach Studieneinschluss wurde bei allen Patienten erfasst.

Statistisch kamen der T-Test bei Normalverteilung sowie der U-Test bei nicht normalverteilten Parametern zur Anwendung (IBM SPSS Statistics, Version 20, Chicago, IL, USA). Die Korrelationsanalysen erfolgten in Abhängigkeit einer vorhandenen oder nicht vorhandenen Normalverteilung mittels Pearson- oder Spearmen-Analyse. Die Bonferroni-Korrektur erfolgte bei multiplen Korrelationen der Biomarker NSE, S100B und NT-proCNP.

Ergebnisse: Zwölf Patienten mit Sepsis (mittleres Alter 67,8±12,1 Jahre) und neun neurologische Kontrollpatienten (mittleres Alter 34,8±13,1 Jahre) wurden eingeschlossen.

Alle Sepsispatienten wiesen klinische Zeichen der SAE, wie Konfusion, Agitiertheit, Halluzinationen oder veränderter Vigilanz auf. Dagegen bot keiner der

neurologischen Kontrollpatienten Zeichen der Enzephalopathie, was passend zu den unauffälligen MR-morphologischen Befunden dieser Patienten war. Die Sepsispatienten zeigten in sechs von neun vorhandenen MRT-Befunden Sepsis-typische Veränderungen der weißen Substanz.

Die Biomarkermessung erbrachte auffällige Unterschiede zwischen den beiden Patientenkohorten. Die NSE-Werte im Plasma waren nicht signifikant unterschiedlich zwischen den beiden Kohorten. Dagegen waren die S100B-Werte der Sepsisgruppe an Tag 1, 3 und 7 jeweils signifikant höher als in der Kontrollgruppe. Auch die NT-proCNP-Werte waren an allen drei Messzeitpunkten jeweils signifikant höher in der Sepsisgruppe und zeigten sich dezelerierend über den Sepsisverlauf. Ein möglicher Hinweis für einen Zusammenhang zwischen systemischer und Neuroinflammation zeigte sich durch eine starke Korrelation der plasmatischen NT-proCNP-Werte und der im Liquor gemessenen NT-proCNP-Level bei Sepsispatienten ($R=0,700$, $p=0,016$).

Bei den Liquorwerten zeigte sich lediglich für die NSE ein signifikant höherer Wert bei Sepsispatienten (8,0±6,0 ng/ml vs. 3,8±2,2 ng/ml, $p<0,05$), wobei die Werte im Normbereich für die NSE lagen. Keine Unterschiede zwischen beiden Kohorten zeigten sich für S100B- und NT-proCNP-Level im Liquor. In der Sepsisgruppe zeigten sich einzig die mittleren NT-proCNP-Level der Patienten mit septischen Hirnläsionen im MRT tendenziell höher als bei Patienten ohne Hirnläsionen (389,2±153,4 vs. 273,8±269,8 pmol/L, $p>0,05$). Auffällig war dagegen eine Korrelation zwischen NT-proCNP- und IL-6-Leveln im Liquor bei Sepsispatienten, die nicht für NSE und S100B nachweisbar war. Ebenso korrelierten die Liquor-NT-proCNP-Level gut mit den Plasma-NT-proCNP-Spiegeln an Tag 1, 3 und 7, was für eine potentielle Verknüpfung zwischen systemischer Inflammation und neuroaxonaler Schädigung angesehen werden kann.

Bei fünf verstorbenen Sepsispatienten zeigten sich keine signifikanten Unterschiede für alle drei Biomarker im Vergleich zu überlebenden Patienten. Der BI war signifikant niedriger in der Sepsisgruppe an Tag 100 (82,1±25,3 vs. 100±0, $p<0,05$), zeigte jedoch keine Korrelation mit den Ergebnissen der Messung aller drei Biomarker.

Schlussfolgerung: Die Messung der plasmatischen NT-proCNP-Level bei Patienten mit Sepsis und SAE könnte sinnvoll sein, um eine neuroaxonale Schädigung sowie das Auftreten einer Enzephalopathie nachzuweisen. Die Korrelation zwischen Liquor- und Plasma-NT-pro-CNP-Spiegeln bei Sepsis sowie die Korrelation mit Biomarkern der systemischen Inflammation, lässt eine Assoziation zwischen systemischer und Neuroinflammation vermuten.

OA 5) Der prognostische Wert der Neurofilament-Level bei Patienten mit Sepsis-assoziierter Enzephalopathie – Eine prospektive Pilotstudie

Ehler, J, Petzold A, Wittstock M, Kolbaske S, Gloger M, Henschel J, Heslegrave A, Zetterberg H, Lunn MP, Rommer PS, Grossmann A, Sharshar T, Richter G, Nöldge-Schomburg G, Sauer M. *The prognostic value of neurofilament levels in patients with sepsis-associated encephalopathy – a prospective, pilot observational study.* PLoS One 2019:14(1):e0211184.

Hintergrund: Die SAE trägt kurz- und langfristig erheblich zum neurokognitiven Outcome der Sepsispatienten bei. In unserer Vorarbeit (**OA 3**) konnten wir neuropathologisch zeigen, dass Neurofilamente auch bei SAE geeignete Biomarker sein könnten, um die SAE prognostisch besser einschätzen zu können.

Fragestellung: Die vorliegende Arbeit sollte erstmalig die Neurofilamente als potentielle Biomarker bei SAE in einer prospektiven Pilotstudie untersuchen.

Material und Methoden: Zwanzig Patienten mit septischem Schock und fünf intensivmedizinisch behandelte Patienten ohne Sepsis und ohne SAE wurden monozentrisch in die prospektive Untersuchung einbezogen. Es erfolgte eine standardisierte multimodale Diagnostik mittels neuropsychiatrischer Untersuchung, EEG, Delir-Screening, cMRT, Liquordiagnostik sowie longitudinalen Plasmauntersuchungen an Tag 1, 3 und 7 nach Studienbeginn zur Messung der Neurofilamente NfL und NfH.

Die statistische Auswertung erfolgte mit der Software SAS (Version 9.4, SAS Institute, Inc., Cary, NC, USA). Normalverteilte Daten wurden mittels T-Test und nicht-normalverteilte wurden mittels Wilcoxon-Test verglichen. Unabhängige Variablen der verschiedenen Studiengruppen wurden mittels ANOVA-Test verglichen. Korrelationsanalysen erfolgten in Abhängigkeit des Vorliegens einer Normalverteilung mittels Pearson- oder Spearman-Test.

Ergebnisse: Insbesondere die Plasma-NfL-Level waren in der Sepsis-Gruppe signifikant höher als bei nicht-septischen Kontrollen ($p=0.0063$) und zeigten einen signifikanten Anstieg zwischen Tag 1 und 7 ($p<0,001$). Bei Patienten mit SAE waren die Plasma-NfL-Werte im Vergleich zu Patienten ohne SAE signifikant höher ($p=0,011$) und zudem durch einen hochsignifikanten Anstieg bis Tag 7 gekennzeichnet ($p<0,001$). Die Plasma-NfH-Level stiegen signifikant zwischen Tag 1 und 7 bei SAE-Patienten an ($p=0,043$). Sepsispatienten mit MR-morphologisch nachweisbaren Hirnläsionen zeigten im Vergleich zu Patienten ohne Läsionen tendenziell höhere Plasma-NfL-Level, zudem war der Anstieg der NfL-Werte über die Zeit bei Patienten mit septischen Hirnläsionen zwischen Tag 1 und 7 signifikant

(p=0,012). Dieser Unterschiede konnte nicht für die Plasma-NfH-Level nachgewiesen werden (p>0,05).

Sechs Patienten, die bis Tag 100 nach Studienbeginn verstorben waren, zeigten zwischen Tag 1 und 7 signifikante Anstiege der Plasma-NfL-Level (p=0,043), die allerdings auch bei Überlebenden nachweisbar waren (p=0,001). Keine Unterschiede zeigten sich bei den Plasma-NfH-Werten (p>0,05). Konträr dazu waren die Liquor-NfL-Level zu Beginn der Sepsis (Liquordiagnostik im Mittel um Tag 3 nach Studienbeginn) bei später verstorbenen Patienten signifikant höher als bei überlebenden Patienten (p=0,012).

Bis Tag 100 nach Studienbeginn zeigte sich eine signifikante Korrelation zwischen NfL im Liquor und dem Barthel-Index an Tag 100 (R=-0,749, p<0,001), was sich auch für die Spitzenspiegel im Plasma nachweisen ließ (R=-0,535, p=0,00003). Es konnte eine Korrelation zwischen Liquor-NfL-Leveln und der Zeitdauer bis zum Versterben beobachtet werden (R=-0,932, p<0,0001), die schwächer ausgeprägt ebenfalls bei den NfH-Werten vorlag (R=-0,657, p=0,011).

Schlussfolgerung: Mit Hilfe dieser Pilotstudie konnten erste Ergebnisse zur Wertigkeit der Neurofilamente NfL und NfH bei Patienten mit SAE präsentiert werden. Die Neurofilamente könnten einen validen Biomarker zur Diagnose, zum Monitoring sowie zur Prognoseabschätzung bei Sepsis und SAE darstellen. Die Poweranalyse zeigte, dass für nachfolgende Hypothesen-basierte Studien deutlich höhere Patientenzahlen nötig sein werden, um zusätzliche Aussagen zur Rolle der Neurofilamente bei neuroaxonaler Schädigung im Rahmen der Sepsis treffen zu können.

DISKUSSION

Diagnostische Methoden zum Nachweis der neuroaxonalen Schädigung im ZNS sind relevant, um nicht nur die Schädigung per se, sondern auch deren Ausmaß zu erkennen und somit das Monitoring- und Therapieregime anpassen zu können.

Die klinische Untersuchung stellt dabei eine wichtige Diagnostik dar, um eine Enzephalopathie diagnostizieren zu können, ist jedoch bei kritisch kranken Intensivpatienten aufgrund häufig notwendiger Analgosedierung und Beatmung nicht immer einfach durchzuführen [75,76]. Daher ist eine erweiterte apparative Diagnostik im Sinne einer zerebralen Bildgebung notwendig, um eine neuroaxonale Schädigung nachweisen zu können [76,81]. Die cMRT ist bei der MS ein fest in den Leitlinien etabliertes Verfahren zum Nachweis demyelinisierender Entzündungsherde im Bereich des Cerebrums und der Spinoaxis und ermöglicht damit die Darstellung der zerebralen Schädigung [38,105,106]. Zusätzlich ist bei der MS mittels Kontrastmittelgabe die Darstellung der Störung der Blut-Hirn-Schranke als Hinweis auf eine akute Entzündungsreaktion möglich **(OA 1 und 2)**, was neben einem möglichst frühzeitigen Behandlungsbeginn auch einen weiteren Prädiktor für den Behandlungserfolg des TPA bei MS darstellen kann (**OA 2,** [107,108]). Bei der SAE ist die cMRT ebenfalls ein wichtiges Diagnostikum, um einerseits Differentialdiagnosen der Enzephalopathie auszuschließen und andererseits eine strukturelle Hirnschädigung nachzuweisen (**OA 3-5**, [81,87,109]. Septische Hirnläsionen sind im Verlauf der Sepsis häufig nachweisbar und könnten eine Ursache für die neurokognitiven Langzeitdefizite von SAE-Patienten sein (**OA 3,** [81,110,111]. Aufgrund des Aufwandes der Methodik und vorhandener Kontraindikationen bei kritisch kranken Patienten auf der Intensivstation wird die cMRT allerdings nicht routinemäßig angewendet [81,83,110]. Dies dürfte die

Häufigkeit des Vorhandenseins zerebraler Läsionen im Rahmen der Sepsis noch unterschätzen. Dennoch ist die cMRT technisch nicht die beste Bildgebung, um detailliert das Ausmaß einer axonalen Schädigung im ZNS nachzuweisen [112-114]. Die Diffusionstensor-Bildgebung (Diffusion Tensor Imaging, DTI) ermöglicht eine detaillierte Darstellung des Faserverlaufs und kann somit die axonale Schädigung und deren Ausprägung noch genauer detektieren [112,115,116]. Weitere Möglichkeiten der zerebralen Bildgebung bei SAE stellen die Positronen-Emissions-Tomographie (PET) und die MR-Spectroskopie dar, die allerdings im Wesentlichen im Rahmen von Forschungsarbeiten und nicht in der täglichen Routine Anwendung finden [116]. Bildmorphologisch ist mit diesen Methoden jedoch keine Aussage über die Funktion einzelner Synapsen sowie über neuronale Plastizität möglich, was sowohl bei der MS als auch bei der SAE limitierend ist.

Von hohem klinischen und wissenschaftlichen Interesse sind sowohl bei der MS als auch bei der SAE Biomarker, die zur Detektion, zum Monitoring und zur Prognoseabschätzung verwendet werden [41,42,89]. Bei der MS wurden diverse Biomarker zur Prädiktion des klinisch nicht vorhersagbaren Erkrankungsverlaufs und zur Beurteilung eines Therapieeffekts verschiedener immunmodulatorischer Therapien verwendet [41,45]. Neurofilamente werden bei der MS nicht nur zur Detektion einer Hirnschädigung, sondern vor allem zur Prognoseabschätzung des Erkrankungsverlaufs und zur Überwachung einer subklinischen Krankheitsprogression verwendet [43,44,117]. Bei der SAE wurden in der Vergangenheit ebenfalls diverse Biomarker untersucht, wobei sich die meisten Studien auf die NSE und das S100B-Protein konzentrierten [89,93,94]. Bisher konnte sich keiner der untersuchten Biomarker in der klinischen Routine etablieren, was mit der unzureichenden axonalen Spezifität dieser Biomarker in Zusammenhang gebracht wird (**OA 4**, [89]. Die bei diversen neurologischen Erkrankungen bereits

intensiv untersuchten Neurofilamente wurden bei der SAE bisher nicht analysiert, scheinen jedoch sowohl im Liquor als auch im Blut von Sepsispatienten zur Detektion und Prognoseabschätzung eine Rolle zu spielen, was in der vorliegenden Arbeit gezeigt werden kann (**OA 3 und 5**). Wir konnten immunhistochemisch im Hirngewebe verstorbener Sepsispatienten, als auch im Liquor und Plasma in vivo bei dieser Patientengruppe signifikant höhere Neurofilament-Spiegel nachweisen, die sowohl mit klinischen als auch bildmorphologischen Befunden der Hirnschädigung korrelierten (**OA 3 und 5**). Interessant war dabei insbesondere, dass zu Beginn der Sepsis bei Patienten und bei nicht-septischen Kontrollen vergleichbare Neurofilament-Spiegel gemessen wurden, die im Gegensatz zu den Kontrollen erst im weiteren Verlauf bei Patienten der Sepsisgruppe anstiegen (**OA 5**). Dieser Anstieg der Neurofilament-Spiegel war bei Patienten mit klinisch nachweisbarer SAE ab Tag 3 nach Sepsisbeginn eindrücklich erkennbar und ließ sich auch bei Patienten mit MR-morphologisch gesehenen septischen Hirnläsionen nachweisen (**OA 5**). Auf Grundlage der Daten unserer Pilotstudie könnten insbesondere die NfL-Spiegel bei Sepsispatienten einen validen Biomarker darstellen, der in Zukunft insbesondere bei klinisch nicht ausreichend evaluierbaren, analgosedierten und beatmeten Patienten dennoch auf eine Hirnschädigung hinweisen würde. Dies könnte im Rahmen einer Nutzen-Risiko-Analyse auch die Indikation zur Durchführung einer zerebralen Bildgebung bei septischen Patienten stärken und somit den Nachweis von strukturellen Hirnläsionen im Rahmen der Sepsis erbringen. Damit hätten die Neurofilamente einen prognostisch relevanten Wert zur weiteren Abschätzung des neurokognitiven Outcomes der Patienten (**OA 5**). Auf Grundlage der Daten unserer Pilotstudie (**OA 5**) scheint, im Gegensatz zum inter-individuellen Wert, vor allem eine Bedeutung für die intra-individuelle Betrachtung der longitudinalen Messung von Neurofilament-Leveln zu bestehen. Ein signifikanter Anstieg der Neurofilament-Level

des Sepsispatienten korrelierte gut mit der im Verlauf der SAE klinisch und bildmorphologisch nachweisbaren ZNS-Schädigung. Zusätzlich konnten wir erstmalig einen prognostischen Wert von NfL-Spiegeln im Liquor bezüglich des harten Endpunktes Überleben bei Sepsispatienten aufzeigen (**OA 5**). Bereits zu einem sehr frühen Beginn der Sepsis (Liquordiagnostik erfolgte im Mittel an Tag 3 nach Studienbeginn) wiesen später verstorbene Patienten signifikant höhere NfL-Spiegel als Überlebende auf, was mit der Zeitdauer bis zum Versterben korrelierte (**OA 5**). Aufgrund der geringen Patientenzahl in dieser Pilotstudie sind weitere Analysen zur Wertigkeit der Neurofilamente notwendig, um deren Relevanz für die Detektion, für das Monitoring sowie die Prognoseabschätzung im Rahmen der Sepsis und SAE weiter zu beleuchten.

Ein weiterer, potentiell für die SAE relevanter Biomarker könnte das NT-proCNP sein, das bei Sepsispatienten mit SAE insbesondere zu Erkrankungsbeginn signifikant im Vergleich zu nicht-septischen Kontrollen erhöht war und somit eine Rolle für die Prädiktion einer SAE spielen könnte **(OA 4)**. Mit den Daten dieser Proof-of-concept-Studie ist durch die gesehenen Korrelationen von klassischen Inflammationsmarkern wie IL-6 und PCT mit dem NT-proCNP eine Verbindung zwischen der systemischen Inflammationsreaktion im Rahmen der Sepsis und der im Verlauf eintretenden Neuroinflammation im Zuge der SAE zu diskutieren (**OA 4**, [118,119]). Es konnten hier tendenziell höhere NT-proCNP-Spiegel bei Patienten mit septischen Hirnläsionen im cMRT nachgewiesen werden, was die potenzielle Nützlichkeit dieses Biomarkers bei SAE unterstreicht (**OA 4**). Da natriuretische Peptide ebenfalls für den Abbau der endothelialen Glykokalix mit der Folge einer erhöhten vaskulären Permeabilität verantwortlich gemacht werden und unsere Daten eine gute Korrelation von Liquor- und Plasma-NT-pro-CNP-Spiegeln aufzeigten, könnte dies indirekt auf eine gestörte Blut-Hirn-Schrankenfunktion hinweisen. Weitere Daten zum NT-

proCNP, auch von septischen Patienten ohne SAE, die wir bisher nicht untersuchen konnten, sind nötig, um die Relevanz für die tägliche klinische Routine abschließend bewerten zu können.

Eine zentrale Frage bei der Durchführung von Biomarkermessungen ist, ob sich aus den Befunden Konsequenzen für das Management der Sepsispatienten in der Intensivmedizin ableiten lassen, da bisher keine spezifischen Therapiemöglichkeiten für die SAE existieren. Es konnten lediglich potenziell reversible Risikofaktoren für die SAE identifiziert werden, deren frühzeitige und suffiziente Therapie das Outcome der SAE möglicherweise verbessern könnte [66]. Da bisher keine einheitlichen Empfehlungen für das Neuromonitoring bei Patienten mit SAE auf Intensivstation existieren ist unklar, ob bei erkennbarem Anstieg eines sensitiven und spezifischen Biomarkers ein intensiviertes Neuromonitoring (z.B. mittels kontinuierlichem TCD, NIRS oder EEG), die neurokognitive Prognose dieser Patienten positiv beeinflussen kann [75,76]. Mit dem bildmorphologischen Nachweis von strukturellen Hirnläsionen im Rahmen der Sepsis wäre allerdings eine präzisere Dokumentation der Hirnschädigung im Rahmen der SAE möglich. Als Konsequenz daraus sollten intensivierte neurologische Rehabilitationsmaßnahmen inklusive neuropsychologischer Therapien im Sinne einer „kognitiven Rehabilitation“ durchgeführt werden [120-122]. Verschiedene Therapieansätze bei Gedächtnisstörungen nach Schädelhirntrauma, Hirnblutung und Schlaganfall existieren bereits und könnten auch bei Patienten mit SAE und neurokognitiven Defiziten regelhaft zur Anwendung kommen. Aufgrund der bisher uneinheitlichen und vermutlich unzureichenden Diagnostik bei SAE auf Intensivstationen führen wir als Konsequenz aus den hier vorgestellten Daten (**OA 3-5**) momentan eine deutschlandweite Online-Befragung durch, wie genau die Diagnostik auf deutschen Intensivstationen bei SAE durchgeführt wird. Da bisher keine suffizienten

Empfehlungen zur Diagnostik und zum Monitoring der SAE existieren, lässt sich aus den Ergebnissen der Befragung ein heterogener „IST-Zustand“ der Diagnostik bei SAE vermuten, der Anlass zur Erarbeitung von konkreten Empfehlungen für die suffiziente diagnostische Abklärung der SAE erbringen sollte.

Im Rahmen der Behandlung akuter MS-Schübe, die als autoimmun-mediiert angesehen werden, kommen immun-suppressive Therapien, wie die GKS-Therapie sowie der TPA zur Anwendung (**OA 1, 2**, [39,50]. Die Gabe von GKS im Rahmen der Sepsistherapie erbrachte keinen Behandlungsvorteil, weshalb deren Gabe nicht routinemäßig empfohlen wird [100,103]. Für den TPA existieren ebenfalls keine Empfehlungen für die routinemäßige Anwendung im Rahmen der Sepsistherapie [100]. Kleinere Studien wurden bei Patienten mit septischem Schock durchgeführt ohne, dass ein klarer Überlebensvorteil gezeigt werden konnte [104,123,124]. Ein nachweisbarer Therapieeffekt des TPA im Rahmen der Behandlung des septischen Schocks war eine schnellere hämodynamische Stabilisierung im Rahmen der Sepsis [104,123,124]. Ob sich das Auftreten und die Dauer neurologischer Komplikationen, wie der SAE, im Rahmen des TPA bei Patienten mit septischem Schock reduzieren lassen, wird unsererseits aktuell im Rahmen einer klinischen Studie untersucht (ClinicalTrials.gov:NCT02906345).

ZUSAMMENFASSUNG UND AUSBLICK

In der vorliegenden Arbeit wurden die MS sowie die SAE als zwei klassische, klinisch und intensivmedizinisch relevante ZNS-Erkrankungen vorgestellt. Beide Erkrankungen zeichnen sich durch eine bisher nicht vollständig verstandene Pathophysiologie aus. Immunologisch vermittelte Pathomechanismen werden bei beiden Entitäten intensiv diskutiert und führen zu akuter Entzündungsaktivität und neuroaxonaler Schädigung im ZNS. Während bei der Autoimmunerkrankung MS die T-und B-Zell-Aktivität zur ZNS-Schädigung führt, scheinen es bei der SAE systemische inflammatorische Mechanismen zu sein, die zu einem sekundären Übergreifen der Inflammation auf das ZNS führen und einen neuroaxonalen Schaden verursachen können. Bei beiden Entitäten sind die klinisch-neurologische Untersuchung, die zerebrale Bildgebung sowie die Biomarkerdiagnostik wichtig, um das Ausmaß der neuroaxonalen Schädigung, den subklinischen und klinischen Verlauf sowie die Prognose der Patienten abschätzen zu können. Die vorliegende Arbeit zeigt dabei den Stellenwert der bereits etablierten Diagnostik mittels klinischer Untersuchung und MRT-Bildgebung zum Nachweis der ZNS-Schädigung am Beispiel der MS auf und präsentiert auf dieser Grundlage neue Daten zum diagnostischen Nachweis der neuroaxonalen Schädigung bei SAE. Im Gegensatz zur MS ist die rein klinische Diagnostik bei Patienten mit Sepsis durch die intensivmedizinische Behandlung häufig erschwert, weshalb bildgebende Verfahren und Biomarker der neuroaxonalen Schädigung größere Relevanz haben. Longitudinale Biomarkerdiagnostik unter Verwendung von Plasma-NfL könnte bei Sepsispatienten zukünftig helfen eine neuroaxonale Schädigung zu detektieren und die Grundlage zur Durchführung einer zerebralen Bildgebung schaffen. Dies könnte

dazu beitragen das diagnostische Management bei SAE auf Intensivstation zu verbessern. Ziel weiterer Hypothesen-basierter Studien soll eine für die klinische Routine praktikable Diagnostik mit hohem Aussagewert für das Ausmaß der neuroaxonalen Schädigung sowie die neurokognitive Prognose der SAE-Patienten sein.

SELBSTSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass ich diese Arbeit selbstständig angefertigt, alle verwendeten Ergebnisse und Daten anderer vollständig aufgeführt und korrekt zitiert habe. Die Mitwirkung Dritter habe ich offengelegt.

Ich versichere weiterhin, dass diese Arbeit nicht zuvor und auch nicht bei einer anderen Fakultät zur Eröffnung eines Habilitationsverfahrens eingereicht wurde.

Ich erkläre hiermit ebenfalls, dass ich die deutsche Staatsbürgerschaft besitze und mir die Habilitationsordnung sowie alle zughörigen Bestimmungen bekannt sind.

Rostock, den 14.06.2019 Dr. med. Johanes Ehler

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Therapeutic Apheresis and Dialysis

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Therapeutic Plasma Exchange in Glucocorticosteroid-Unresponsive Patients With Clinically Isolated Syndrome

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Abstract: Clinically Isolated Syndromes (CIS) summarize clinical features of possible multiple sclerosis (MS) as a first clinical event of the disease. Escalation therapy in CIS episodes comprises high dose glucocorticosteroid (GCS) treatment followed by therapeutic plasma exchange (TPE) in patients unresponsive to GCS. The aim of our study was to analyze TPE effects in CIS patients. Eleven GCS-unresponsive patients exhibiting CIS were treated with TPE. A median of 5.0 (range 3–8) treatments were performed with a median exchange volume of 3.0 L (range 2.2–3.5 L). Standard diagnostic results in CIS patients were collected. In 10 out of 11 patients clinical improvement was observed. In Expanded Disability Status Scale (EDSS) Scoring, a commonly used score to assess disability in MS and CIS patients, significant improvement was shown as well. One patient was a non-responder to TPE. Apheresis treatments were well tolerated in all patients. In the medical control of GCS-unresponsive CIS episodes, TPE appears to be an effective and well-tolerated treatment option. TPE response in CIS patients is comparable to TPE results in GCS-unresponsive MS relapses. Further prospective studies are indicated. **Key Words:** Apheresis, Escalation therapy, Multiple sclerosis, Neuroimmunology, Steroid-refractory.

Clinically Isolated Syndromes (CIS) represent clinical features of multiple sclerosis (MS) as a first acute or subacute episode of disease (1). Diagnostic criteria of MS, as dissemination in time and space, however, are not achieved at the time of presentation (2). The extent of clinical symptoms in CIS depends on the location of white matter lesions and its effect on functional systems (3). Cranial and spinal magnetic resonance imaging (MRI) is crucial in showing structural lesions typical for MS and to exclude other differential diagnoses (4).

In 60–70% of CIS patients, cerebrospinal fluid (CSF) analysis shows intrathecal IgG synthesis represented by a pathologic IgG-index or oligoclonal bands (OCB) (4,5). This finding is associated with high risk of progression to MS (5).

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Neurophysiologic examination of evoked potentials (EPs) is an additionally used diagnostic tool in CIS and MS (6). EPs provide quantitative information on the functional integrity of motoric, somatosensoric and visual pathways (MEP, SSEP, VEP, respectively) of the central nervous system (CNS) (6). They can be of value by identifying clinically silent brain lesions and are associated with an increased risk of progression to MS as well (7).

There are different clinical tests to assess neurologic impairment in CIS and MS patients (8). Standardized neurologic examination during scheduled visits and patients notifying clinicians about a change in symptoms are important for clinical evaluation and to measure treatment response (8).

In CIS episodes and acute MS relapses, escalation therapy recommendations comprise glucocorticosteroid (GCS) pulse treatment with 1 g methylprednisolone (MP) given daily over 3–5 days. If definite improvement is not achieved within 2 weeks after completion of the first GCS treatment a higher second GCS pulse (up to 2 g MP given daily over 3–5

days) can be considered (9). If clinical improvement cannot be achieved again within 2 weeks after completion of the second GCS treatment, therapeutic plasma exchange (TPE) is recommended (10).

These recommendations are based on clinical responses observed in different studies, although the exact mechanisms of action of TPE are still unknown (11–15). Therapeutic effects of TPE in CIS and MS patients are suspected to depend on different regulatory effects in the immune system, finally resulting in immunosuppression (11). Disease activity is localized in the CNS. Depletion of inflammatory factors such as antibodies, immune complexes and cytokines in plasma and peripheral nervous system (PNS) are known to result from TPE (11). Hence, TPE effects are supposed to result from a breakdown of supply of inflammatory factors involved in the CNS inflammatory process (11,16). A direct impact of TPE on immune cells is suspected as well (17).

Different studies analyzed TPE effects in acute MS and chronic progressive disease courses (10,11,18). Based on literature and therapeutic guidelines, TPE can be performed in acute MS relapses and CIS episodes but is not effective in chronic progressive MS as a disease modifying therapy strategy (10,19).

Clinical experience and actual studies with TPE focusing on CIS patients are very rare in the literature. Only a few specialized MS centers published their own experiences with small numbers of mainly MS and only few CIS patients included. Patients treated by TPE, however, responded well in these studies (13–15,20). Based on CIS definition the first ever clinical episode of possible emerging MS was treated by TPE in GCS-unresponsive patients. The aim of our study was to analyze TPE effects in CIS focusing on the clinical response and further development of our CIS patients.

PATIENTS AND METHODS

Patients

We retrospectively evaluated the data of 11 CIS patients treated by TPE (Table 1). Eligible subjects for analysis were CIS patients without achievement of clinical and Expanded Disability Status Scale (EDSS) improvement by GCS treatment (i.e. GCS-unresponsive).

Clinical evaluation of CIS patients

The EDSS, commonly used to assess disability in MS (21), was used for clinical evaluation and scoring before, during and after TPE. Based on eight functional systems (pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, other) the EDSS is a 10-point scale of disease severity between 0 (no disability) and 10 (death) and is used to assess clinical outcome and disease progression (8,21). EDSS-scoring was performed by EDSS-certified neurologists.

Definition of TPE response (8,22,37) was primarily based on changes of the predominant neurological symptom (functional system) by clinician's examination and by notification of the patient (primary endpoint). Marked improvement was defined as clinically significant improvement in function, whereas mild improvement represented slight but definite improvement without functional gain. Additionally, EDSS change during treatment was used to measure TPE response (secondary endpoint). TPE response was defined as EDSS decrease ≥1.0 points in patients

TABLE 1. *Plasma exchange therapy in Clinically Isolated Syndrome (CIS) patients*

Patient/Gender	Age (years)	Predominant symptom at TPE (FS)	Initiation of TPE (days)	No. TPE	TPE until effect	EDSS before initiation of TPE	EDSS after completion of TPE	EDSS at follow-up
1/F	20	Cerebellar	61	5	3	3.0	2.0	n.a.
2/M	29	Brainstem	60	5	5	3.0	2.0	2.0
3/F	22	Myelitis	10	8	8	3.0	2.5	2.0
4/F	41	Pyramidal	91	5	5	5.5	4.5	5.5
5/M	20	Pyramidal	31	6	4	7.5	6.0	6.0
6/F	24	Myelitis	31	5	5	2.0	1.0	1.0
7/F	38	Pyramidal	61	3	3	6.5	4.5	4.0
8/F	29	Sensory	90	5	5	3.0	1.5	1.5
9/M	30	Optic neuritis	61	5	5	1.5	1.0	1.0
10/F	27	Cerebellar	120	3	3	2.5	2.5	2.5
11/F	28	Cerebellar	94	8	8	2.0	1.0	1.0
Median (range)	28.0 (20–41)		61.0 (10–120)	5.0 (3–8)	5.0 (3–8)	3.0 (1.5–7.5)	2.0 (1.0–6.0)	2.0 (1.0–6.0)

EDSS, expanded disability status scale; F, female; FS, functional system (Kurtzke); M, male; n.a., not assessed; No., number; TPE, therapeutic plasma exchange.

TABLE 2. *Patient and technical characteristics of plasma exchange therapy*

Patient	Body weight (kg)	Vascular access	Exchange volume (L)	TPE platform	Anticoagulation
1	64	PV	2.5	PF 1000	UFH
2	74	PV	2.2	PF 1000	UFH
3	57	PV	3.0	L 18/ PF 1000	Citrate + UFH
4	76	CVA	3.0	PF 1000	UFH
5	64	PV→CVA	3.0	PF 1000	UFH
6	71	CVA	2.5	PF 1000	UFH
7	75	PV	3.0	L 18	Citrate + UFH
8	110	CVA	3.5	PF 1000	UFH
9	84	CVA	3.5	L 18	Citrate + UFH
10	64	PV	2.5	PF 1000	UFH
11	102	CVA	3.0	PSU 25	UFH
Median (range)	74.0 (57–110)		3.0 (2.2–3.5)		

CVA, central venous access; L 18, Life18 base system with therapeutic plasma exchange set (Miltenyi Biotech, Bergisch-Gladbach, Germany); PF 1000, Baxter BM 11/14 (Baxter Healthcare, Deerfield, USA) with Gambro PF1000 plasma filter (Gambro AB, Lund, Sweden); PSU25, Fresenius Multifiltrate with plasma filter PSU25 (Fresenius Medical Care AG, Bad Homburg, Germany); PV, peripheral vein; TPE, therapeutic plasma exchange; UFH, unfractionated heparin.

with initial EDSS ≤ 5.5 or EDSS decrease ≥0.5 points in patients with EDSS ≥ 6.0 before initiation of TPE (22).

Results of MRI, EPs and CSF, achieved before initiation of GCS treatment and TPE, were obtained by standard diagnostic methods according to guidelines in CIS and MS (2,6,23). Follow-up examinations were performed shortly after TPE in our in- or outpatient department.

TPE

All 11 patients gave informed consent before the beginning of TPE (Table 2). The plasma volume was estimated using nomograms (24). A minimum of three TPE sessions per patient was planned with an interval of 2 days between procedures. Based on experience and kinetic considerations at least three TPE sessions should be performed for a reduction of circulating IgG of up to 70% (15,25). Further treatments up to a maximum of eight TPE sessions (2 days between procedures) followed depending on change of the patient's predominant neurologic symptom assessed by daily clinical examination.

In every treatment session a single plasma volume was exchanged (3.0 L in median, range 2.2–3.5 L). One liter of the replacement solution contained 750 mL Ringer's lactate solution according to Hartmann (Ringer-Lactat nach Hartmann, B. Braun Melsungen AG, Melsungen, Germany) and 250 mL of human serum albumin 20% (Alburex 20%, CLS Behring GmbH, Marburg, Germany). The albumin concentration in the ready to use fluid was 5%.

Therapeutic plasma exchange procedures were performed via peripheral veins in six patients and central venous access in five patients (Table 2). Different machine settings (platforms) have been used for TPE (Table 2). Anticoagulation with unfractionated heparin (standard regime with heparin bolus of 1000–2000 U at the beginning of TPE, followed by 500–800 U/h) was monitored by the activated clotting time (ACT; 180–200 s during anticoagulation, Hemochron 401, ITC, Edison, NJ, USA). Using the Miltenyi Life18 system for TPE a heparin bolus of 2000 U was administered, followed by citrate anticoagulation (citrate:inlet flow ratio 1:22, using Anticoagulant Citrate Dextrose Solution A [ACD-A]). According to the manufacturer's presetting, a calcium replacement was not necessary in the Life18 system. In patients symptomatic for hypocalcemia, ionized calcium was measured. These patients were treated with 10 mL of 10% calcium gluconate solution (B. Braun Melsungen AG, Melsungen, Germany) to normalize systemic calcium levels (reference range 1.0–1.2 mmol/L).

The inlet flow rate was 80–100 mL/min and the average plasma flow rate was 20–25 mL/min in treatments with peripheral vascular access. In treatments with central venous access the inlet flow rate was 150 mL/min with an average plasma flow rate up to 35 mL/min, respectively.

Statistics

Comparison of continuous variables of EDSS scores before and after TPE as well as at time of follow-up was performed using non-parametric tests (Friedman and Wilcoxon Tests). Statistical significance was indicated by $P < 0.05$ (IBM SPSS Statistics, Version 20, Chicago, IL, USA).

TABLE 3. *Diagnostic findings in magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) in Clinically Isolated Syndrome (CIS) patients before therapeutic plasma exchange (TPE)*

Patient	MS-like lesions (MRI)	Gd-lesions (MRI)	Cell count Mpt/L (CSF)	Protein mg/L (CSF)	OCB (CSF)	IgG-Synthesis (CSF)
1	+	–	3	283	+	–
2	+	–	2	446	+	–
3	+	–	7	397	–	–
4	+	+	1	371	–	–
5	+	+	5	272	+	+
6	+	–	21	380	+	+
7	+	+	5	381	+	n.a.
8	+	–	3	308	+	–
9	+	+	7	785	+	+
10	+	–	2	343	+	–
11	+	+	2	400	+	+
Median (range)			3 (1–21)	380 (272–785)		

+, detected; –, not detected; Cell count reference range in CSF ≤ 5 Mpt/L; Gd, gadolinium contrast medium; IgG, immunoglobulin G; MRI, magnetic resonance imaging (combined brain and spinal cord); MS, multiple sclerosis; n.a., not assessed; OCB, oligoclonal bands; Protein reference range in CSF = 150–450 mg/L.

RESULTS

Patient demographics

Between February 2001 and May 2012 11 GCS-unresponsive CIS patients were treated by TPE. The cohort consisted of eight female and three male patients with a median age of 28 years (range 20–41 years). An immunosuppressive medication was non-existent in the patients' history.

The patients' demographic and neurologic characteristics are shown in Table 1.

Diagnostic findings

Results of diagnostic findings of MRI and CSF in CIS patients before TPE are shown in Table 3 and EP results are shown in Table 4.

TABLE 4. *Results of evoked potentials in Clinically Isolated Syndrome (CIS) patients before therapeutic plasma exchange (TPE)*

Patient	MEP	SSEP	VEP
1	+	+	–
2	–	–	+
3	+	+	–
4	+	+	–
5	+	+	+
6	–	–	–
7	+	+	+
8	–	+	–
9	+	+	+
10	+	+	+
11	–	–	–

MEP, motor-evoked potentials; SSEP, somatosensory-evoked potentials; VEP, visual-evoked potentials; +, pathologic result (loss of potential or prolonged latency or definite site difference); –, physiologic result.

GCS treatment before TPE

All patients were treated with 1 g MP given daily over 5 days. Without achievement of definite improvement within 14 days after completion of the first MP treatment, a second MP pulse of 2 g MP was given daily over 5 days in 10 out of 11 patients (90.9%). One patient (patient 3) developed a fulminant transverse myelitis and received TPE directly after completion of the initial treatment with 1 g MP.

In 9 out of 11 patients (81.2%), no clinical improvement was observed after completion of MP treatment. One patient (9.1%) achieved slight but definite improvement without functional gain (mild improvement; patient 5).

Two patients (18.2%) developed adverse events associated with MP treatment. One patient complained of gastrointestinal symptoms (nausea and stomach pain), another patient developed allergic exanthema.

Clinical data at the time of GCS treatment are shown in Table 5 and Figure 1a.

TPE

All 11 CIS patients received an initial TPE series. Median time from beginning of symptoms to start of TPE was 61.0 days (range 10–120 days). A median of 5.0 TPE sessions were performed (range 3–8 treatments).

Primarily based on clinical assessment, 10 out of 11 patients (90.9%) showed improvement of the predominant symptom by TPE (Fig. 1b). Two out of 11 patients (18.2%) presented marked clinical improvement. In 8 out of 11 patients (72.7%) mild clinical improvement was observed. No TPE effect (patient 10) could be seen in one patient (9.1%). In patients

TABLE 5. *Glucocorticosteroid treatment in Clinically Isolated Syndrome (CIS) patients*

Patient	CMP dose (g)	Main symptom at MP treatment (FS)	Effect of MP treatment	Adverse events
1	15	Cerebellar	No effect	None
2	15	Brainstem	No effect	Gastrointestinal
3	5	Myelitis	Deterioration	None
4	15	Pyramidal	No effect	Allergy
5	15	Pyramidal	Mild improvement	None
6	15	Myelitis	No effect	None
7	15	Pyramidal	No effect	None
8	15	Sensory	No effect	None
9	15	Optic neuritis	No effect	None
10	15	Cerebellar	No effect	None
11	15	Cerebellar	No effect	None

CMP, cumulative methylprednisolone; FS, functional system; MP, methylprednisolone.

with clinical response to TPE ($N = 10$), improvement was documented after 5.0 TPE sessions in median (range 3–8 sessions).

Based on quantitative EDSS measurement and our secondary TPE response definition, 8 out of 11 patients (72.7%) were TPE responders. In 3 out of 11 patients (27.3%), no response to TPE was observed according to EDSS response definition.

Significant EDSS improvement comparing EDSS score before (median EDSS 3.0, range 1.5–7.5) and after TPE (median EDSS 2.0, range 1.0–6.0) was seen ($P = 0.004$).

All TPE sessions were well tolerated by our CIS patients. Due to insufficient blood flow, one patient needed to be switched from peripheral to central venous access (patient 5). Severe adverse events associated with TPE, as defined as failed vein puncture, catheter infection, hematoma or thrombosis did not occur in our patients. Most patients showed a slight decrease of blood pressure at the beginning of

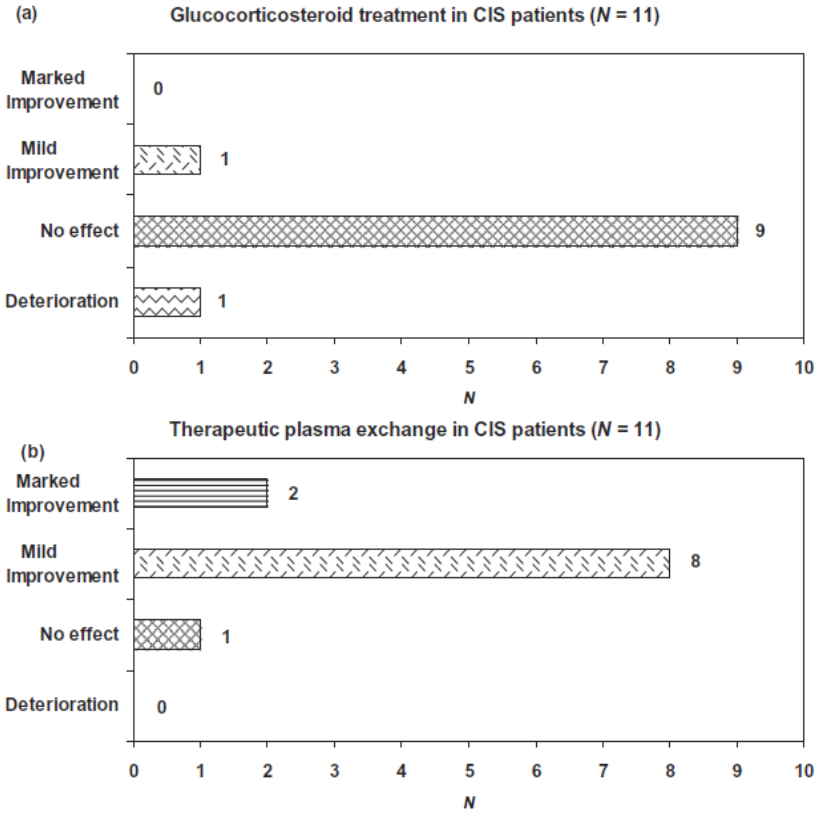

FIG. 1. (a) Response to glucocorticosteroid treatment in Clinically Isolated Syndrome patients. (b) Response to therapeutic plasma exchange in Clinically Isolated Syndrome patients.
Deterioration, clinical symptom deteriorated and/or additional symptoms; Marked Improvement, clinically significant improvement in function; Mild Improvement, slight but definite improvement without functional gain; *N*, number of patients; No Effect, clinical symptoms unchanged.

TPE sessions without treatment consequences. Temporary moderate hypotension (systolic blood pressure below 95 mm Hg) without clinical symptoms occurred in one patient and was treated by crystalloid infusion (no ACE inhibitor in the patient's history).

Details of TPE are shown in Table 1 and Figure 1b.

Follow-up examination after TPE

Ten out of 11 patients (90.9%) could be subjected to follow-up examinations and were given medical care exclusively at our MS center. One patient did not continue follow-up in our clinic after TPE was finished (patient 1).

Patients were seen again after a median of 71.5 days (range 1–187 days) after their final TPE session for clinical re-evaluation and EDSS scoring (median EDSS 2.0, range 1.0–6.0).

During follow-up examination ($N = 10$), 1 out of 9 TPE responders (patient 4, EDSS 5.5) did show deterioration of the main symptom and EDSS increase towards baseline EDSS again. In all other TPE responders re-evaluated in follow-up (8 out of 9), clinical and EDSS improvement were confirmed. Two of these TPE responders even showed further EDSS decrease during follow-up (patient 3 EDSS 2.0, patient 7 EDSS 4.0).

Relapses and long-term follow-up

Four of 10 CIS patients developed a second episode after their final TPE session (median duration 136.5 days, range 1–277 days). During the following GCS treatment ($N = 3$) with 1 g MP, in all three patients definite clinical improvement (i.e. GCS response), was observed. One patient (patient 3), diagnosed with neuromyelitis optica (NMO), immediately received another TPE session.

During long-term follow-up 8 out of 10 CIS patients developed relapsing-remitting MS (80%), one patient NMO (10%) and one patient (10%) did not show any further disease activity to date. In 8 out of 10 patients (80%), a disease modifying or immunosuppressive therapy after TPE was established (Table 6).

TABLE 6. *Disease modifying therapy in Clinically Isolated Syndrome (CIS) patients after therapeutic plasma exchange (TPE) (*N = *10)*

Disease modifying drug	*N*
Interferon beta 1-a	1
Interferon beta 1-b	1
Mitoxantrone	1
Natalizumab	2
Glatirameracetate	1
Intravenous immunglobulin	1
Rituximab	1
No treatment	2

DISCUSSION

Based on the general hypothesis of the depletion of inflammatory mediators, TPE is recommended in MS and CIS relapses today (10,26). Even direct impact on immune cells with possible modifications of neuroimmunologic mechanisms in MS or CIS are discussed (11,17,27). Unfortunately, after decades of intensive research exact mechanisms of MS and TPE are still poorly understood (11).

Most MS patients develop a chronic course of disease over time. An underlying amplifying immunologic process, resulting in chronification, is discussed (28). Assuming similar immunologic mechanisms in CIS and MS, chronification or amplification should not have occurred prior to a first episode of CIS. Therefore, TPE with a focus on this early stage of disease may be of interest.

In our CIS patient population we observed excellent response rates with 91% of partial or even complete clinical improvement and these results are in line with previous reports exhibiting beneficial effects of TPE in 87% of MS patients (15). Other studies demonstrated good clinical response in up to 93% of patients with MS or other CNS demyelinating diseases (11,13,14,16,37). Response rates to TPE in the majority of available studies; however, were derived from an inhomogeneous patient population including patients suffering from MS, CIS, NMO or optic neuritis. To date, a comparable TPE series focusing on CIS patients is non-existent. In contrast to other studies, data from TPE in the present study are derived from patients exhibiting CIS only and may therefore supplement the pre-existing literature with novel insights of TPE as a beneficial therapeutic option.

Even due to high response rates in this study, one patient did not show clinical improvement by TPE. A switch from TPE to immunoadsorption therapy with selective elimination of autoantibodies performed in other studies may be an alternative for some patients (29). We agree with Trebst et al. that prospective randomized studies comparing both techniques should be conducted (15,29).

Interestingly, not all CIS patients develop MS (4,5,23). Hence, further prospective studies focusing on long-term outcome after initial TPE in CIS should be engaged. In reference to MS literature, disease modification by TPE could not be observed. Therefore, TPE is not recommended as a disease

modifying therapy (9,10,19,30). Reliable studies concerning further CIS development after initial TPE are non-existent.

As MS lesions have been differentiated into four immunopathologic patterns (31), Keegan et al. (32) demonstrated TPE response in relation to pattern II (antibody/complement-associated demyelination), suggesting humoral mechanisms in patients with inflammatory demyelinating diseases. Surprisingly some of our primarily GCS-unresponsive patients developed GCS response after TPE. Furthermore, superimposed relapses during TPE of a previous GCS-unresponsive MS relapse have been successfully treated by GCS (33). From our point of view these observations demonstrate the insufficiently understood mechanisms of TPE on the disease processes in CIS and MS. Pure elimination of antibodies and complement factors cannot explain all clinical TPE effects (33). Heterogeneous patterns of demyelination or different stages of damaged brain tissue, which respond differently to TPE and GCS, might be a possible explanation (33,34). Additionally, the impact on immune cells and possible cell changes (e.g. lymphocytes) by apheresis treatment have been discussed and should be analyzed in further studies (17,35).

Time from the beginning of symptoms to the start of TPE has also been of interest to many authors in the literature (13–15,36). Keegan et al. recommended an early initiation of TPE in MS patients within 6 weeks after symptom onset to achieve TPE response (13). Prediction of clinical improvement by an early start of TPE was documented by Llufriu et al. as well (36).

Although initiation of TPE was around 9 weeks after onset of symptoms in median in our patients, clinical improvement was still demonstrated. Results from Trebst et al. (15) as well as Meca-Lallana et al. (14) agree with our results of positive improvement, even after a later initiation of treatment. We agree with Trebst et al. that patients with severe relapses should be considered for TPE independently from their duration of symptoms (15).

In our retrospective analysis TPE response could be verified by clinical assessment and EDSS rating at the end of TPE as well as in follow-up examination. Based on current knowledge TPE responders improve immediately after TPE (11,15). In contrast, the patients' neurological status at the time of follow-up probably was influenced by TPE effects and the impact of the disease modifying therapy that was established after TPE.

Objective diagnostic evaluation of clinical symptoms and therefore clinical treatment effects in CIS and MS are hard to achieve in some patients. Due to often polysymptomatic clinical presentation and diagnostic limitations to definitely measure somatasensoric and motoric dysfunction (e.g. by EP), accurate clinical assessment and EDSS rating seemed to be the only reliable factors to evaluate TPE response in our series (21). The use of clinical examination to define TPE responses can be seen as a non-objective evaluation method. Hence, quantitative EDSS rating would be favorable, but is limited by insufficient symptom representation (e.g. optic neuritis or different extents of somatosensoric dysfunction). Therefore, different TPE response rates were observed between clinical (91% responders) and EDSS (73% responders) assessment in our series and do reveal the accurate clinical response definition to be beneficial.

Small patient numbers and a missing control group without TPE are main limitations of this retrospective analysis. Based on current knowledge, sham-treatment in GCS-unresponsive patients at the end of escalation therapy today cannot be justified ethically due to the good response rates observed in severely affected CIS patients (9,11,13–16,37). A randomized trial ($N = 22$) from Weinshenker et al. did show beneficial effects of TPE in comparison to sham-treatment in a heterogeneous group of CNS demyelinating diseases in 1999 (37). Current sham-controlled trials focusing on CIS patients are missing.

CONCLUSION

Clinically Isolated Syndrome patients responded well to therapeutic plasma exchange. At the end of escalation therapy, therapeutic plasma exchange appears to be a promising option in glucocorticosteroid-unresponsive CIS episodes. Based on available data from literature and our own results, therapeutic plasma exchange may play an important role in the medical control of CIS. Further prospective studies with therapeutic plasma exchange and immunoadsorption treatment in CIS patients with appropriate patient numbers are encouraged.

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RESEARCH ARTICLE

Response to Therapeutic Plasma Exchange as a Rescue Treatment in Clinically Isolated Syndromes and Acute Worsening of Multiple Sclerosis: A Retrospective Analysis of 90 Patients

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Abstract

Objectives

Experience with therapeutic plasma exchange (TPE) for acute relapses in clinically isolated syndrome (CIS) or multiple sclerosis (MS) patients has been derived from small and inhomogeneous patient populations so far. In the present study, we retrospectively evaluated features associated with TPE response in a larger cohort of CIS and MS patients with acute worsening of disease.

Participants

Ninety CIS and MS patients with acute relapses or acute worsening of symptoms were firstly treated with TPE. The population consisted of 62 women and 28 men with a median age of 38 years (range 18–69 years).

Outcome Measures

Primary endpoint was the clinical response to TPE, focused on the functional improvement of the target neurologic deficit. Secondary endpoint was an improvement in expanded disability status scale (EDSS) scoring.

Results

A clinical response to TPE was observed in 65 out of 90 patients (72.2%), with marked improvement in 18 (20.0%) and moderate improvement in 47 out of 90 patients (52.2%). The median EDSS was reduced from 3.75 before to 3.0 after TPE ($p = 0.001$). Response to TPE was significantly more frequent in patients with relapsing courses of disease (CIS,

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RR-MS, $p = 0.001$), no disease modifying drugs ($p = 0.017$), gadolinium-positive (Gd^+) MRI lesions ($p = 0.001$) and EDSS ≤ 5.0 before TPE ($p = 0.014$). In the multiple logistic regression analysis only the detection of Gd^+ MRI lesions was significantly altered ($p = 0.004$).

Conclusion

Clinical response to TPE was achieved in the majority of our patients. We identified clinical and diagnostic features in CIS and MS relapses that might be helpful to identify patients responding to TPE. Gd^+ MRI lesions before treatment were the best predictor of the response to TPE in our cohort.

Introduction

Multiple sclerosis (MS) is an immune mediated disease of the central nervous system (CNS) significantly causing proceeding disability in young adults [1–3]. The underlying mechanisms of this relapsing and often chronic progressive disease are insufficiently understood [4, 5]. Modern immunomodulating strategies, however, offer several therapeutic approaches to clinically isolated syndrome (CIS) and MS patients today [6–8]. First line treatment of acute relapses is high dose glucocorticosteroid (GCS) pulse treatment with initially 1g methylprednisolone (MP), given daily over 3–5 days. A higher second GCS pulse with up to 2g MP can be considered in unresponsive patients after an interval of 2 weeks [9–12]. If symptoms persist despite GCS treatment, the relapse is defined as GCS-unresponsive and therapeutic plasma exchange (TPE) is recommended [7, 9, 10, 13]. Meanwhile, TPE is implemented into therapeutic guidelines of a broad spectrum of neurological disorders [7, 14–17]. The elimination of humoral factors (antibodies, complement factors, cytokines and immune complexes), all assumed to be involved in inflammation and demyelination in CIS and MS, is currently regarded as the rationale for TPE [7, 18, 19]. Beneficial TPE effects have been detected in about 40–90% of patients with acute relapses [14, 16, 20–25]. Male sex, preserved reflexes and an early initiation of treatment were associated with successful TPE [22, 23, 25, 26]. The response to TPE in the majority of pre-existing inhomogeneous studies was derived from patients with optic neuritis (ON), neuromyelitis optica (NMO), MS and CIS [15–17, 22, 23, 25, 26]. Studies focusing on MS or CIS included predominantly small patient numbers of 4–60 patients each [14–16, 20–27].

Therefore, the aim of our study was to analyse the response to TPE in a larger population consisting of 90 CIS and MS patients.

Materials and Methods

Participants and inclusion criteria

Based on the 2001 Mc Donald criteria [28], TPE data were evaluated from 90 patients diagnosed with acute worsening of CIS or MS from February 2001 to June 2013. The study was approved by the local ethics board at Rostock University (identifier A 2015–0065). All patient information and records were anonymised and de-identified prior to analysis. Of 11 included CIS patients treated between 2001 and 2012, data were previously analysed and published recently [14].

Inclusion criteria were unresponsiveness to GCS treatment or pre-existing contraindications for the use of GCS and no previous TPE in the patients' history [9, 10]. Deteriorated,

unchanged and insufficiently improved symptoms (slight change in symptom without impact on function) after GCS treatment were defined as GCS-unresponsiveness. An acute relapse was defined as a new and definite clinical attack. According to this definition secondary-progressive (SP)-MS patients with superimposed relapses were included. Primary-progressive (PP)-MS patients with a clinical worsening of neurologic function were evaluated as well.

Outcome measures

The Expanded Disability Status Scale (EDSS) was used for clinical evaluation and scoring before, during and after GCS treatment and TPE by EDSS-certified neurologists [29]. The response to treatment was analysed at the end of each patient's final TPE session. Follow-up examinations were performed in our in- or outpatient department. Routinely, the interval to the clinical re-evaluation after TPE was 3 months at our centre.

a) Primary endpoint of the response to TPE. Definition of the response to treatment was primarily based on changes of the predominant neurological symptom (target neurologic deficit, assigned to a functional system) by clinician's examination and by patient's notification [17, 22, 30, 31]. Marked improvement was defined as clinically significant improvement in function. Moderate improvement represented a definite change of the neurologic deficit without significant impact on function within the functional score. No effect comprised unchanged symptoms. Deterioration represented patients with worsened target neurologic deficit or new neurologic symptoms.

b) Secondary endpoint of the response to TPE. Additionally, EDSS change during treatment was used to measure GCS effects and the response to TPE. Response was defined as an EDSS decrease $\geq$1.0 points in patients with an initial EDSS $\leq$5.5 or an EDSS decrease $\geq$0.5 points in patients with an initial EDSS $\geq$6.0 [32].

Diagnostic procedures before TPE

Available data from MRI and motor-, somatosensory- and visual-evoked potentials (MEP, SSEP and VEP, respectively), were obtained employing standard diagnostic methods according to CIS and MS guidelines before the initiation of GCS treatment [28, 33]. The results are summarised in Table A in S1 File.

TPE procedures

All 90 patients gave written informed consent for TPE and central venous access before the initiation of treatment. The plasma volume was estimated using nomograms [34]. A minimum of 3 TPE sessions per patient was determined with an interval of 2 days between procedures. Based on experience and kinetic considerations at least 3 TPE sessions should be performed for a reduction of circulating IgG of up to 70% [25, 35]. In severely affected patients, the first 2 TPE sessions were performed daily. Further treatments up to a maximum of 8 TPE sessions (2 days between procedures) were performed, if either none or only mild positive changes of the patient's predominant neurologic symptom were seen. In every treatment session, a single plasma volume was exchanged (2.8 L in median, range 1.9–4.0 L). One litre of the replacement solution contained 750 mL Ringer lactate solution according to Hartmann (Ringer-Lactat nach Hartmann, B. Braun Melsungen AG, Melsungen, Germany) and 250 mL of human serum albumin 20% (Alburex 20%, CLS Behring GmbH, Marburg, Germany). The albumin concentration in the ready to use fluid was 5%. Patients with known coagulation disorders or patients with a high bleeding risk were treated with fresh frozen plasma instead of albumin solution. TPE procedures were performed via peripheral veins in 31 patients and via central venous access in 55

out of 90 patients. In 4 patients vascular accesses were switched from peripheral veins to central venous access due to failed vein puncture.

Different machine settings (platforms) were used for TPE (Fresenius Multifiltrate with plasma filter PSU2S, Fresenius Medical Care AG, Bad Homburg, Germany; Baxter BM 11/14, Baxter Healthcare, Deerfield, USA with Gambro PF1000 plasma filter, Gambro AB, Lund, Sweden; Miltenyi Life18 system with therapeutic plasma exchange set, Miltenyi Biotech, Bergisch-Gladbach, Germany). The inlet flow rate was 80–100 mL/min and the average plasma flow rate was 20–25 mL/min in treatments with peripheral vascular access. In treatments with central venous access the inlet flow rate was 150 mL/min with an average plasma flow rate of up to 35 mL/min.

Statistical analysis

Univariate comparison of baseline characteristics concerning the response to TPE was performed using Pearson's Chi-Square test for independent variables.

Comparison of continuous variables of EDSS values before and after TPE as well as at time of follow-up was performed using non-parametric tests (Wilcoxon-Test). Multiple logistic regression analysis (stepwise forward) was used to assess the effect of baseline and treatment variables on TPE response (disease modifying drugs (DMD) before TPE, number of previous relapses, time between GCS and TPE treatment, cumulative GCS dosage, symptom worsening during GCS treatment, EDSS before TPE, time to TPE, gadolinium-positive (Gd^+) lesions in MRI). Statistical significance was indicated by $p < 0.05$ (IBM SPSS Statistics, Version 20, Chicago, IL, USA).

Results

Clinical characteristics and diagnostic findings before the initiation of TPE

Between February 2001 and June 2013 a total of 90 CIS and MS patients were treated with TPE. The patient population consisted of 62 female (68.9%) and 28 male patients (31.1%) and comprised patients with CIS, relapsing-remitting MS (RR-MS), SP-MS with superimposed relapses and PP-MS with acute worsening of disease (Table 1).

A single functional system (monosymptomatic relapse) was affected in 15 out of 90 patients (16.7%) and multiple functional systems (polysymptomatic relapse) in 75 out of 90 patients (83.3%). In polysymptomatic relapse patients the predominant neurological symptom (target neurological deficit) was determined and assigned to the respective functional system to analyse the response to TPE. Out of 90 patients the affected functional systems were: visual function in 10, brainstem function in 10, sensoric function in 10, myelitis in 17, cerebellar function in 16, pyramidal function in 24 and mental function in 3 patients, respectively. In 28 out 90 patients (31.1%) a symptom progression within a functional system or an extension to other functional systems was observed during GCS treatment. DMD before TPE were present in 47 out of 90 patients (52.2%, Table B in S1 File). Details of baseline characteristics and diagnostic findings are shown in Tables A and B in S1 File.

GCS treatment before the initiation of TPE

Eighty-one out of 90 patients received a high dose GCS treatment prior to TPE (Fig 1A). In 9 out of 90 patients, GCS were not administered to treat the acute relapse due to documented GCS-non-response during previous relapse treatment (n = 4) and due to known severe adverse events (steroid-induced pancreatitis, femoral head osteonecrosis with fracture) against GCS

Table 1. Patient characteristics.

Characteristics	CIS	RR-MS	SP-MS	PP-MS	All patients
No. of patients	21	46	18	5	90
Age (yrs)[a]	32.0 (20–63)	37.5 (18–56)	42.0 (23–69)	47.0 (24–66)	38.0 (18–69)
Body weight (kg)[a]	75.0 (50–109)	67.5 (47–128)	65.0 (49–106)	70.0 (50–95)	69.5 (47–128)
Disease duration (mo)[a]	2.0 (1–5)	56.5 (1–273)	171.0 (3–249)	37.0 (10–158)	47.0 (1–273)
DMD (%)	0	60.9	83.3	60.0	51.1
Previous relapses[a]	1.0 (1)	3.0 (1–20)	10.0 (2–30)	3.0 (1–5)	3.0 (1–30)
Relapses per year[a]	1.0 (0.5–2.0)	1.0 (0–4)	0.9 (0.2–9.0)	1.0 (0.4–3.0)	1.0 (0–9)

CIS = clinically isolated syndrome, DMD = disease modifying drug, MS = multiple sclerosis, PP-MS = primary progressive MS with acute worsening, RR-MS = relapsing-remitting MS, SP-MS = secondary progressive MS with superimposed relapse.

[a] Median (range).

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(n = 2). In three patients with an intermittent GCS treatment (1g MP given daily over 5 days every 3 months) the new clinical attack was evaluated as GCS-unresponsive and these patients immediately received TPE.

The median EDSS before GCS treatment was 3.75 (range 1.0–8.5). Median duration from the beginning of symptoms to the start of GCS treatment was 5.0 days (range 1–61 days). In 55 out of 81 patients (67.9%) GCS treatment comprised a pulse with 1 g MP/die and a following pulse with 2 g MP/die (ultra-high dose GCS treatment). Twenty-six out of 81 patients (32.1%) were treated merely with a pulse of 1 g MP/die due to rapid disease progression or adverse

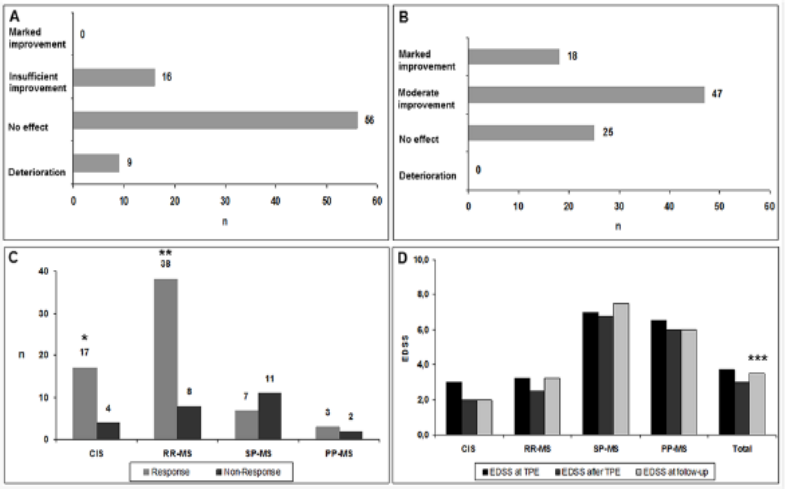

Fig 1. Glucocorticosteroid treatment and therapeutic plasma exchange in clinically isolated syndrome and multiple sclerosis patients. A) Response to glucocorticosteroid treatment in CIS and MS patients (n = 81). B) Response to therapeutic plasma exchange in CIS and MS patients (n = 90). C) Different responses to therapeutic plasma exchange in CIS and MS patients (n = 90). D) Development of median EDSS values during therapeutic plasma exchange (n = 90). CIS = clinically isolated syndrome, Deterioration = clinical symptom worsened and/or additional symptoms, Insufficient improvement = slight change in symptom without impact on function, Marked improvement = clinically significant improvement in function, Moderate improvement = definite change of the neurologic deficit without significant impact on function within the functional score, MS = multiple sclerosis, n = number of patients, No effect = clinical symptom unchanged, PP-MS = primary-progressive MS with acute worsening, RR-MS = relapsing-remitting MS, SP-MS = secondary-progressive MS with superimposed relapse, * $p = 0.01$ (CIS versus SP-MS), ** $p = 0.002$ (RR-MS versus SP-MS), *** $p = 0.001$ (EDSS before versus after TPE).

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Table 2. Therapeutic plasma exchange in clinically isolated syndrome and multiple sclerosis patients.

	CIS	RR-MS	SP-MS	PP-MS	All patients
No. of patients	21	46	18	5	90
Time to TPE (days)[a]	50 (2–145)	60.5 (7–154)	61.0 (18–163)	99.0 (7–152)	57.5 (2–163)
No. of TPE[a]	6 (2–8)	5 (3–8)	5 (2–8)	5 (3–5)	5 (2–8)
TPE until effect[a]	5 (2–8)	5 (2–8)	5 (2–7)	3 (3–5)	5 (2–8)
Clinical response to TPE[a]	17 (81%)	38 (82.6%)	7 (38.9%)	3 (60.0%)	65 (72.2%)
EDSS before TPE[a]	3.0 (1.0–8.5)	3.25 (1.0–6.5)	7.0 (5.5–8.5)	6.5 (3.0–7.5)	3.75 (1.0–8.5)
EDSS after TPE[a]	2.0 (0.0–8.0)	2.5 (1.0–6.5)	6.75 (5.5–8.5)	6.0 (2.5–7.5)	3.0 (0.0–8.5)
EDSS at follow-up[a]	2.0 (0.0–7.5)	3.25 (1.0–7.0)	7.5 (4.5–8.5)	6.0 (3.0–8.0)	3.5 (0.0–8.5)

CIS = clinically isolated syndrome, EDSS = expanded disability status scale, MS = multiple sclerosis, No. = number, PP-MS = primary progressive MS with acute worsening, RR-MS = relapsing-remitting MS, SP-MS = secondary progressive MS with superimposed relapse, TPE = therapeutic plasma exchange.

[a] Median (range).

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events related to GCS. The median cumulative dosage of GCS was 15.0 g MP per patient (range 3–15 g). Fifteen out of 81 patients (18.5%) complained adverse events during GCS treatment (gastrointestinal in 5, dermatologic in 3, cardiovascular in 3 and psychiatric in 4 patients, respectively).

Response to TPE

All 90 patients received an initial TPE-series (Fig 1B–1D, Table 2). Median time from the beginning of symptoms to the start of TPE (time to TPE) was 57.5 days (range 2–163 days).

Based on our clinical response definition, responses to TPE were observed in 65 out 90 patients (72.2%). Marked improvement were seen in 18 (20.0%) and moderate improvement in 47 out of 90 patients (52.2%). The response to TPE was documented after a median of 5.0 single TPE sessions (range 2–8 sessions). Twenty-five out of 90 patients (27.8%) were non-responder to TPE. Regarding our EDSS response definition, 31 out 90 patients (34.4%) were responder and 59 out of 90 patients (65.6%) were unresponsive to TPE. A significant EDSS decrease ($p = 0.001$) between median EDSS values before (3.75, range 1.0–8.5) and after TPE (3.0, range 0.0–8.5) was observed (Fig 1D, Table 2).

Analysis of TPE-efficiency

All clinical and diagnostic baseline variables were analysed regarding to the response to TPE. In the univariate statistical analysis, patients without a pre-existing medication of DMD ($p = 0.017$) and without worsening during GCS treatment ($p = 0.043$) showed a significant response to TPE. Beneficial TPE was significantly more frequent in CIS ($p = 0.010$) and RR-MS ($p = 0.002$) patients as in SP-MS patients. In direct comparison of relapsing (CIS, RR-MS) and progressive disease (SP-MS, PP-MS), response to TPE was significantly more frequently observed in relapsing courses ($p = 0.001$). Moreover, response to TPE was significantly more often observed in patients with a baseline EDSS ≤ 5.0 ($p = 0.014$). In contrast to T_2 lesions in MRI, the detection of Gd^+ lesions was strongly associated with the response to TPE ($p = 0.001$). Gender, age, number of previous relapses, time to TPE, affected functional systems and pathological evoked potential results were independent from the response to TPE. In addition, there was no significant difference between patients treated with a pulse of 1 g MP/die and patients treated with 1g and 2g MP/die before TPE (Table C in S1 File).

Based on the completely available data in 70 of our patients (MRI exams were not available in 13 and a cumulative MP dose in 9 patients; 2 of these patients had neither MRI nor MP), a multiple logistic regression model was conducted. Gd^+ lesions in MRI ($p = 0.004$) emerged as the only factor predicting the response to TPE in our patients (Table 3).

Adverse events of TPE

In 25 out of 466 single TPE-procedures (5.4%) adverse events occurred in 23 out of 90 patients (25.6%, 2 patients had two adverse events each). None of our patients died during the observation time. Severe adverse events were allergic reactions during TPE in three patients (mild allergic reaction to fresh frozen plasma in 1 patient, allergy to albumin in 1 patient, unknown cause of allergy in 1 patient, respectively) and a systemic infection with septic shock after the third TPE session in 1 patient. For this case, TPE was interrupted for intensive care treatment and was finished after the recovery. In 1 case with symptomatic segmental pulmonary embolism during the second session, TPE was interrupted for the treatment with low molecular heparin (Table 4). In 14 out of 67 patients (20.9%) with relapsing disease courses (CIS, RR-MS), adverse events occurred. In patients with progressive disease courses (SP-MS, PP-MS), adverse events were detected in 4 out of 23 patients (17.4%). The differences between relapsing and progressive disease groups were not significant ($p = 0.488$).

Follow-up examination and further relapses after TPE

Eighty-seven out of 90 patients (96.7%) could be subjected to follow-up examinations after TPE at our MS centre. With a median time of 62 days (range 9–353 days) after their final TPE session, patients were re-evaluated for clinical and EDSS scoring (median EDSS at follow-up 3.5, range 0.0–8.5). The comparison of EDSS values at the end of the final TPE session (EDSS 3.0) and at the time of follow-up (EDSS 3.5) showed a non-significant increase in EDSS ($p = 0.256$). In 35 out of 87 patients (40.2%) a new relapse was observed during long-term follow-up (observation deadline: June 2013). The median time to relapse after their last TPE session was 101 days (range 4–2487 days). The median EDSS was 4.5 (range 1.0–9.0).

At the end of long-term follow-up, 2 patients were diagnosed with CIS (2.3%), 58 with RR-MS (66.7%), 20 patients with SP-MS (23.0%), 6 with PP-MS (6.9%) and 1 patient with NMO (1.2%).

Table 3. Multiple logistic regression analysis of clinical baseline variables in clinically isolated syndrome and multiple sclerosis patients (n = 70).

Independent variable	OR	95% CI	*p*
Time to TPE	0.994	0.977–1.011	0.485
DMD before TPE	0.419	0.085–2.072	0.286
Gd^+ lesions in MRI before TPE	**0.095**	**0.019–0.479**	**0.004**
Number of relapses before TPE	1.093	0.915–1.306	0.325
EDSS before TPE	0.95	0.616–1.466	0.815
Cumulative MP dose before TPE	0.647	0.259–1.617	0.352
Time to GCS treatment before TPE	1.042	0.995–1.092	0.082
Worsening during GCS treatment	0.391	0.066–2.325	0.302

CIS = clinically isolated syndrome, DMD = disease modifying drug, EDSS = expanded disability status scale, GCS = glucocorticosteroid, Gd^+ = gadolineum contrast medium enhancement, MP = methylprednisolone, MRI = magnetic resonance imaging, MS = multiple sclerosis, TPE = therapeutic plasma exchange.

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Table 4. Adverse events during therapeutic plasma exchange (n = 466 single sessions).

Adverse event	No.	Treatment
Local infection due to vascular access	1	Local antiseptics
Local bleeding due to vascular access	3	Local compression
Pain due to vascular access	1	Peripheral analgetics
Electrolyte imbalance (hypocalcaemia)	1	10% calcium gluconate
Temporary paresthesia	1	No treatment
Allergic reaction	3	Antihistamines + Prednisolone
Moderate hypotension	2	Crystalloid infusion
Coagulation imbalance	4	Specific substitution
Dislocation of peripheral vascular access	1	Change of access
Failed puncture for central venous access	1	No treatment
Vasovagal syncope	1	Symptomatic treatment
Systemic infection without sepsis	4	Antibiotics
Systemic infection with sepsis	1	Sepsis treatment on ICU
Thrombosis and pulmonary embolism	1	Anticoagulation
Total adverse events	**25**	

ICU = intensive care unit, Moderate hypotension = systolic blood pressure below 95 mmHg, No. = number.

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Discussion

Previous TPE studies [16, 17, 20, 22] focused on acute exacerbations of different CNS inflammatory diseases (CIS, MS, ON or NMO). Therefore, clinical experience was derived from inhomogeneous patient populations. Pre-existing studies predominantly included a small number of patients [14, 16, 17, 20–22]. Current prospective clinical trials focusing on TPE in CIS and MS are non-existent. Hence, systematic evaluations of clinical variables and its associations with the response to TPE are of interest for daily clinical routine.

The results of TPE from our study were derived from a population of 90 patients with MS spectrum disorders (CIS and MS), the great majority of them was GCS-unresponsive. Our finding of about 72% of responders to TPE corresponds with previous reports and could supplement the pre-existing literature with new aspects [22, 23, 25].

In order to measure response to TPE accurately, we primarily focused on the changes in a target neurological deficit and secondarily in the EDSS. Quantitative EDSS rating is supposed to be favourable in comparison to subjective clinical examination of single symptoms. Otherwise, pure evaluation of EDSS values is limited by insufficient symptom representation in this score and could indicate response to TPE imprecisely [14]. Accordingly the differences between our clinical (72% responders) and our EDSS (34% responders) response definitions reveal, that the accurate clinical response definition is better suitable for the treatment with TPE. Some limitations of our study have to be mentioned. The lack of a control group to compare our results with TPE and the retrospective character were limitations of our analysis. Other limitations were the lack of routine timing of treatment and of evaluation after TPE, heterogeneous treatment receiving groups and the unblinded assessment of the primary outcome. A prospective randomised trial performed to compare true and sham TPE in CIS and MS would be best to evaluate TPE effects and is warranted. As long as TPE represents the last therapeutical option for GCS-unresponsive patients, a clinical trial with patients assigned to a sham treatment cannot easily be justified due to the beneficial effects of TPE observed in available studies [14, 17, 22, 23, 25, 36, 37]. A randomisation of GCS-unresponsive patients into a cohort treated merely by GCS and patients additionally treated with TPE would end up in the

same ethical dilemma. Although adverse events occurred in the minority of our patients, various risks of an extracorporeal treatment are further contraindications for sham TPE [38]. The relatively small number of TPE complications in our patients could be underestimated due to the retrospective assessment of adverse events. Bleeding complications through anticoagulation or adverse events through central venous puncture (e.g. thrombosis, infection or vascular damage) can occur and are unacceptable for sham treated patients at the current stage of experience with TPE in CIS and MS [38, 39]. To date, both the exact mechanisms of CIS and MS and the mechanism of the influence of TPE on these diseases are still not clear and need further research [40, 41]. In contrast to the disease activity of CIS and MS localised in the CNS, the depletion of inflammatory factors with TPE in plasma is achieved in the peripheral immune system [19, 40]. Therefore, effects of TPE are supposed to result from a breakdown of supply of immunologic factors maintaining CNS inflammation [14, 24, 40]. Pure elimination of autoantibodies and inflammatory mediators cannot explain all effects of TPE [19]. A direct impact on immune cells in MS and CIS is discussed [19, 40, 42]. In 2005, Keegan et al reported on favourable responses to TPE in relation to immunopathologic pattern II (antibody/complement-associated demyelination) and treatment failure in patients with pattern I and III [43]. These findings supported the hypothesis of humoral mechanisms of action in MS and TPE and should explain responses to TPE more easily. Complementary to Keegan's observations, heterogeneous patterns of demyelination and different stages of damaged brain tissue are meanwhile supposed to respond differently to TPE and GCS [32, 43, 44]. The different responses to TPE-rates among the various MS types underline our deficits to explain effects of TPE in these patients. In the present study response to TPE-rates varied significantly between relapsing forms of disease (CIS, RR-MS) and progressive disease courses (SP-MS, PP-MS). An underlying amplifying immunologic process and irreversible axonal damage in the course of MS are discussed in these cases [45]. This may result in the chronification of the disease and could explain the worse response to TPE in SP-MS and PP-MS, but on the other hand the better results of TPE in CIS and RR-MS patients [45, 46].

Response to TPE was significantly more common in patients without the administration of DMD in our study. It can be hypothesized, that mechanisms of TPE may be more effective in an immune system only influenced by GCS. DMD interfere with various immune system components and could reduce the effectiveness of TPE in these patients. Certainly, no DMD was found predominantly in CIS and RR-MS patients with a greater response to TPE. We were unable to differentiate the impact of TPE with or without DMD and of different MS types in our study, as SP-MS and PP-MS patients almost all received DMD.

Beneficial TPE was significantly associated with a baseline EDSS ≤ 5.0 in the present study and goes along with observations from Magana and Keegan et al [15, 22]. EDSS values of > 5.0 were observed predominantly in the SP-MS and PP-MS group in our study and represented mainly chronic progressive patients with severe neurologic impairment. Neurodegeneration might predominate neuroinflammation and could explain the worse response to TPE in these patients. Consistent with previous studies in chronic progressive MS, beneficial effects of TPE were significantly fewer in SP-MS and PP-MS patients [47].

Interestingly, the time from relapse onset to the start of TPE did not influence the response rate significantly. In the majority of studies, time to treatment within 6 weeks was considered most relevant for treatment success [15, 22, 26]. Median time to TPE was around 8 weeks in our cohort, but beneficial effects of TPE were demonstrated in about 72% of patients. Axonal damage and irreversible neuronal impairment are known to develop most likely during prolonged CNS inflammation [22]. Therefore, a short time to treatment should be pursued to interrupt inflammation and response to TPE is achievable even after longer periods [14, 22]. Especially in patients with a severe neurological deficit, TPE should be considered

independently from the duration of symptoms [14, 25]. The inflammatory intensity, the extent of Gd^+ MRI lesions and individual disease stages at the time of TPE might influence the response to TPE more likely than a short time to treatment [14, 23, 25].

In line with observations from Magana et al successful TPE was related to Gd^+ lesions in MRI in our study [15]. As Gd^+ lesions represent blood brain barrier disruption and do reflect a state of acute CNS inflammation, mechanisms of TPE could be more effective in these patients [23]. Recently, Meca-Lallana et al investigated effects of TPE on radiologic resolution of ring-enhancing MS lesions in MRI [23]. In contrast to the importance of Gd^+ lesions before TPE, the resolution of active lesions after TPE was not associated with response to TPE and did not correlate with the patients prognosis [23].

Conclusion

In comparison to pre-existing small sample studies, response to TPE was verified in a larger cohort of 90 CIS and MS patients. Beneficial treatment effects in about 72% of patients and few adverse events, confirm the important role of TPE in the medical control of CIS and MS relapses. Gd^+ lesions in MRI were the best predictor of the response to TPE in our study. TPE response was significantly more common in patients with relapsing (CIS, RR-MS) than in progressive disease courses (SP-MS, PP-MS). Other potential predictors of the response to TPE, as time from relapse onset, age, gender or the administered GCS dosage before TPE were independent from treatment success. Prospective clinical trials focusing on TPE in CIS and MS patients are encouraged.

Supporting Information

S1 File. Diagnostic findings, disease modifying drugs and univariate statistical analysis results. Diagnostic findings before the initiation of treatment (**Table A**). Administration of disease modifying drugs before the initiation of therapeutic plasma exchange (n = 47) (**Table B**). Univariate statistical analysis of therapeutic plasma exchange in clinically isolated syndrome and multiple sclerosis patients (**Table C**).
(PDF)

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Author Contributions

Conceived and designed the experiments: JE UKZ. Performed the experiments: JE UKZ SK SM HH. Analyzed the data: JE UKZ MS. Contributed reagents/materials/analysis tools: SK SM HH. Wrote the paper: JE UKZ SK MS. Critical revision of the manuscript for important intellectual content: JE UKZ SK MS SM HH RB.

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Translational evidence for two distinct patterns of neuroaxonal injury in sepsis: a longitudinal, prospective translational study

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Abstract

Background: Brain homeostasis deteriorates in sepsis, giving rise to a mostly reversible sepsis-associated encephalopathy (SAE). Some survivors experience chronic cognitive dysfunction thought to be caused by permanent brain injury. In this study, we investigated neuroaxonal pathology in sepsis.

Methods: We conducted a longitudinal, prospective translational study involving (1) experimental sepsis in an animal model; (2) postmortem studies of brain from patients with sepsis; and (3) a prospective, longitudinal human sepsis cohort study at university laboratory and intensive care units (ICUs). Thirteen ICU patients with septic shock, five ICU patients who died as a result of sepsis, fourteen fluid-resuscitated Wistar rats with fecal peritonitis, eleven sham-operated rats, and three human and four rat control subjects were included. Immunohistologic and protein biomarker analysis were performed on rat brain tissue at baseline and 24, 48, and 72 h after sepsis induction and in sham-treated rats. Immunohistochemistry was performed on human brain tissue from sepsis nonsurvivors and in control patients without sepsis. The clinical diagnostics of SAE comprised longitudinal clinical data collection and magnetic resonance imaging (MRI) and electroencephalographic assessments. Statistical analyses were performed using SAS software (version 9.4; SAS Institute, Inc., Cary, NC, USA). Because of non-Gaussian distribution, the nonparametric Wilcoxon test general linear models and the Spearman correlation coefficient were used.

Results: In postmortem rat and human brain samples, neurofilament phosphoform, β-amyloid precursor protein, β-tubulin, and H&E stains distinguished scattered ischemic lesions from diffuse neuroaxonal injury in septic animals, which were absent in controls. These two patterns of neuroaxonal damage were consistently found in septic but not control human postmortem brains. In experimental sepsis, the time from sepsis onset correlated with tissue neurofilament levels ($R = 0.53$, $p = 0.045$) but not glial fibrillary acidic protein. Of 13 patients with sepsis who had clinical features of SAE, MRI detected diffuse axonal injury in 9 and ischemia in 3 patients.

Conclusions: Ischemic and diffuse neuroaxonal injury to the brain in experimental sepsis, human postmortem brains, and in vivo MRI suggest these two distinct lesion types to be relevant. Future studies should be focused on body fluid biomarkers to detect and monitor brain injury in sepsis. The relationship of neurofilament levels with time from sepsis onset may be of prognostic value.

Trial registration: ClinicalTrials.gov, NCT02442986. Registered on May 13, 2015.

Keywords: Intermediate filaments, Biomarkers, Animal models, Rats, Sepsis, Encephalopathy, Sepsis-associated encephalopathy, SAE

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Background

Sepsis still carries a high incidence and mortality rate [1, 2]. Furthermore, sepsis-associated encephalopathy (SAE) in survivors can result in long-term physical, cognitive, and psychological impairment with high socioeconomic relevance [2, 3]. The condition is thought to be underrecognized [4, 5]. Current understanding of pathophysiological mechanisms of SAE is limited; more insights into this important field are needed [6, 7]. Animal models can shed some light on the complex pathophysiological mechanisms of SAE [8–10]. Microglial activation, mitochondrial dysfunction, oxidative stress, neuroinflammation, and neuronal apoptosis are potential risk factors for SAE, resulting in axonal degeneration [11–13]. The accumulation of β-amyloid peptide (Aβ), a part of the β-amyloid precursor protein (βAPP), forms neurotoxic amyloid plaques in the septic brain [14, 15]. The detection of misfolded proteins in the septic brain was linked to long-term cognitive deficits in rats [14]. Histologic demonstration of axonal injury by βAPP staining is now widely used in animal models and human neuropathology [16–18]. Beside advances in histology, in vivo diagnostics can detect axonal injury in SAE [19, 20]. Imaging and biomarker studies have been used to detect brain injury and predict neurologic outcome [21–23]. Nevertheless, both the primary mechanisms underlying SAE and the temporal development of SAE over the course of sepsis remain elusive [24]. We thus conducted a translational study to compare neuropathologic findings derived from postmortem brain samples of septic rats and humans with in vivo clinical and imaging results from patients with sepsis who had SAE. A correlation between newly formed septic brain lesions detected by neuroimaging with neuropathologic findings may provide better understanding of the temporal relationship between sepsis and the onset of SAE.

Methods

Rat model of sepsis-associated encephalopathy

All experiments were performed according to local ethics committee (University College London) and Home Office (UK) guidelines under the 1986 Scientific Procedures Act. Adult male Wistar rats (approximate body weight 300 g, 12–14 weeks old) were used to generate a 3-day in vivo sepsis model of fecal peritonitis. Sepsis induction and experimental procedures were performed as described in detail before [25]. Four naive (noninstrumented) rats, eleven sham-operated, and fourteen septic rats were included. All rats were instrumented under brief anesthesia with tunneled carotid arterial (left side) and jugular venous (right side) catheters to monitor hemodynamics, sample blood, and infuse fluids. The catheters were mounted onto a swivel-tether system, allowing the rat, on recovery from anesthesia, to have free movement in its cage and ad libitum access food and water. Sepsis was induced 24 h later by an intraperitoneal injection of fecal slurry (0.63 mg/100 g body weight, prepared from bowel contents of rats from the same batch). From 2 h postinsult, fluid resuscitation using a 1:1 solution of colloid and 5% glucose was administered at a rate of 10 ml/h for the first 24 h and then halved on successive days to ensure normovolemia and normoglycemia. All septic animals showed clinical signs of sepsis by decreased movements, decreased alertness, hunched posture, and piloerection from about 12 h postinjection of fecal slurry. Rats were killed by cervical dislocation under deep isoflurane anesthesia either 24, 48, or 72 h after sepsis induction. After craniectomy, brains were removed within a few minutes of death. The brain was dissected through the midline into halves. One half was snap-frozen in liquid nitrogen; the other half was placed in formalin.

Histology, immunohistochemistry, and protein extraction from septic rat and human brain

Neuropathologists (MG, FS) were masked to the condition of rat and human brain samples. Human and rat brain tissue from frontal lobe areas and the cerebellum were processed in paraffin wax using a standard 7-day dehydration and paraffin-embedding protocol on an automated tissue processor. Five-micrometer paraffin sections were cut, mounted onto Superfrost™ glass slides and dried overnight at 37 °C. For the general histologic examination, sections were dewaxed, rehydrated, and stained with H&E using a standard protocol. For the immunohistochemical detection of neurofilaments (Nf) and glial fibrillary acidic protein (GFAP), the sections were dewaxed and rehydrated before being placed in 600 ml of 0.1 M citrate buffer (pH 6.0) and microwaved at full power for 15 minutes in a 850-W microwave oven. The four sections were allowed to cool before being rinsed in 0.05 M PBS, pH 7.4, and incubated overnight at room temperature in either a mouse monoclonal antibody directed against an epitope common to the 70 and 200 kDa Nf proteins (clone 2 F11 diluted 1:20; MP Biomedicals Inc., Santa Ana, CA, USA), β-tubulin (1:200; Sigma-Aldrich, Gillingham, UK), β-APP (1:500; Dako, Ely, UK), or a rabbit polyclonal antibody directed against GFAP (diluted 1:1500; Dako). After being washed in PBS, the sections were incubated in a biotinylated secondary antibody for 1 h (Dako), followed by a washing step and incubation in peroxidase-conjugated streptavidin for 1 h (diluted 1:300; Sigma-Aldrich) All dilutions were in PBS with 0.1% Triton-X. Antibody localization was visualized by incubating the sections for 10 minutes at room temperature in 0.05% diaminobenzidine with 0.04% $NiCl_2$ and 0.01% hydrogen peroxide added. The sections

were then counterstained with hematoxylin, dehydrated, cleared, and mounted.

The dry weight of the snap-frozen rat brain tissue was 0.56 to 1.94 g. Barbitone ethylenediaminetetraacetic acid (EDTA) buffer (pH 9.6) containing a protease inhibitor cocktail (P8340; Sigma-Aldrich) was added to 1:2 wt/vol. The samples were homogenized on ice using an ULTRA-TURRAX T 25 instrument (IKA-Werke GmbH & Co., Staufen, Germany) for 1 minute, followed by sonication on ice for 1 minute. Samples were refrozen at −70 °C and then thawed at 30 °C, and 2 ml of sample was added to 5 ml of diisopropyl ether and 2 ml of barbitone EDTA buffer. After a mixing step, the samples were spun at 25,000 × *g* and 4 °C for 30 minutes, and the protein soluble fraction was collected. Tissue levels of Nf heavy chain (NfH^{SMI35}) and GFAP were measured by enzyme-linked immunosorbent assay (ELISA), and total protein was measured using the Lowry method [26, 27].

In vivo neurologic assessment of patients with sepsis

The study was approved by the local ethics board at Rostock University (A 2012-0058) and registered as a clinical trial (ClinicalTrials.gov, NCT02442986). The patient recruitment period was from November 2012 to May 2015. All patients or their legal representatives signed written informed consent forms before study inclusion. Inclusion criteria for participants were aged ≥ 18 years and the presence of severe sepsis or septic shock according to the criteria used at the time [28]. Exclusion criteria were preexisting cerebrovascular diseases, including dementia, preexisting neuromuscular disease, high-dose glucocorticoid administration (>300 mg hydrocortisone or equivalent per day), preexisting renal replacement therapy, coagulopathy with active bleeding, and frequent administration of neuromuscular blocking agents (more than three times per week). Twenty patients with septic shock were included prospectively in the study. Seven participants without magnetic resonance imaging (MRI) examinations were excluded for the following reasons: death before MRI performed ($n = 1$), only cranial computed tomographic scan available owing to contraindication for MRI ($n = 2$), disclaimer for MRI from patient/legal representative after study inclusion ($n = 2$), and repeated surgery/unstable patient ($n = 2$). In total, 13 patients were enrolled prospectively in this single-center, longitudinal, observational study.

Clinical assessment protocol

All patients were clinically assessed by an interdisciplinary team consisting of intensivists and neurologists experienced in critical and neurocritical care using a validated scales for severity of disease: Acute Physiology and Chronic Health Evaluation II at ICU admission and the Sepsis-related Organ Failure Assessment score [29, 30]. All patients received standardized management according to guideline recommendations [2, 28]. After study inclusion, patients were longitudinally evaluated (study days 1, 3, 7, and 28) for their level of consciousness and for signs of SAE, such as confusion, agitation, hallucinations, or acute changes in mental status using the Glasgow Coma Scale, the Richmond Agitation-Sedation Scale, and the Confusion Assessment Method in the Intensive Care Unit (CAM-ICU) [31–34]. A medical history was taken from all patients, if obtainable, or from their next of kin. A standardized neurologic examination was performed on all patients by an experienced neurologist (MW). This comprised a detailed status of the level of consciousness; brainstem reflexes and function; deep tendon reflexes; and sensory and motor function, including muscular strength testing using the Medical Research Council dyspnea scale score [35, 36].

Electroencephalography

In addition to clinical assessment, all patients underwent electroencephalography (EEG) within the first 72 h after sepsis was diagnosed. The international 10–20 system was used for standard electrode placements with impedance level < 5 kΩ on a mobile EEG unit (ED 14; Madaus Schwarzer, Munich, Germany). All EEG recordings were done over 30 minutes. Patients were stimulated by verbal command. If no response to verbal stimulation could be obtained, sternal rub or nail bed compression were performed. The EEG recordings were assessed by an experienced accredited reader (MW) according to the method described by Young et al. [37]. Patients with analgosedation (standard regimen with continuous infusion of propofol and sufentanil) had a sedation holiday of 30 minutes before EEG recording.

Magnetic resonance imaging

A 1.5-T magnet system (MAGNETOM Avanto; Siemens Healthcare, Erlangen, Germany) was used in seven patients, and a 3.0-T magnet system (MAGNETOM Verio; Siemens Healthcare) was used in six patients. A standardized MRI protocol was used, and all MRI findings were analyzed by an experienced neuroradiologist (AG). MRI examinations included coronal T1-weighted images (with and without contrast medium), sagittal and axial T2-weighted sequences, axial fluid-attenuated inversion recovery (FLAIR), and axial T2*-weighted gradient recalled echo sequences. Additionally, axial echo planar imaging diffusion-weighted imaging (DWI) sequences, apparent diffusion coefficient maps, and time-of-flight magnetic resonance angiography were performed. The extent of white matter hyperintensities (WMH) as an imaging marker of brain injury was graded on a previously validated scale. WMH were scored according to

their number and size from grade 0 (no lesions) through grade 1 (punctiform), grade 2 (patchy or confluent), and grade 3 (diffuse) [21, 38]. An MRI examination was performed as soon as the patient was clinically stable for in-house transfer using continuous patient monitoring (Expression MR400 monitor; Phillips Healthcare Deutschland GmbH, Hamburg, Germany).

Statistical analysis

All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Inc., Cary, NC, USA). Because of non-Gaussian distribution, the nonparametric Wilcoxon test was used for comparing two independent variables. Two-way unbalanced analysis of variance (general linear model) was used for comparing more than two independent variables, followed post hoc analysis if significance was achieved. The *F* values providing the degrees of freedom and the number of samples included in each particular analysis are shown. The linear correlation between continuous variables was evaluated using the Spearman correlation coefficient. Linear regression analysis was performed using the least squares method. A p value < 0.05 was considered significant.

Results

Experimental sepsis in rats

Average total protein levels were comparable between groups (naive 7.4 ± 2.6 g/L, sham 8.7 ± 3.9 g/L, and sepsis 8.9 ± 2.9 g/L). We found that brain tissue levels for GFAP were not statistically different when we compared sham-treated (0.27 ± 0.19 ng/g total protein) and septic (0.29 ± 0.21 ng/g total protein) rats with naive rats (0.34 ± 0.11 ng/g total protein). Average brain tissue levels of NfH^{SMI35} were higher in sham-treated (2.6 ± 2.2 ng/g total protein) and septic (1.8 ± 1.7 ng/g total protein) rats than in naive rats (0.8 ± 0.6 ng/g total protein), but this difference failed to reach statistical difference ($p = 0.094$ and $p = 0.356$, respectively) (Table 1). In septic rat brain tissue, there was a mild correlation between NfH^{SMI35} levels and time from sepsis induction ($R = 0.53$, $p = 0.045$) that was not seen for either GFAP ($R = -0.39$, $p = 0.154$) or total protein ($R = -0.05$, $p = 0.854$). No such correlations were observed in either sham-treated or naive rat brain samples. The immunohistochemistry of brain tissue from septic compared with naive and sham-treated rats showed two types of lesions. The normal white matter appearance in a sham-treated rat brain is shown in Fig. 1a. Typically, βAPP staining is restricted to the neuronal cell soma and the proximal axonal hillock. For comparison, in septic brain tissue, diffuse, more widespread axonal staining is seen (Fig. 1b). This pathologic staining was most marked for long white matter tracts (Fig. 1c). Spinal tissue was not available. The second lesion type seen comprised scattered small ischemic lesions (Fig. 1d). The early inflammatory component suggests a septic embolic etiology. These pockets of ischemic, inflammatory lesions stained intensely for βAPP, extending peripherally from the core lesion (Fig. 1e). An alternative marker for early axonal injury is β-tubulin. In sham-treated rats, the axonal staining for β-tubulin is neat and continuous, as expected on the basis of preserved neuronal (Fig. 1f) and axonal (Fig. 1g) integrity. This is best appreciated in the magnified insets in Fig. 1g and h. In contrast to the diffuse axonal pathology present in septic rat brains, axonal β-tubulin is subjected to proteolysis and broken up, giving the impression of a structurally disorganized axonal (Fig. 1h) and neuronal cytoskeleton (Fig. 1i).

Human postmortem sepsis brain study

Postmortem brain tissue was available from five patients who died as a result of sepsis (mean age 64 years, one male patient). The three control subjects (mean age 37 years, all male) died as a result of other causes: suicide, assault, and road traffic accident (RTA) without concurrent brain injury.

Causes leading to death in sepsis were directly related to multiorgan failure, but one patient had additional complications in the form of a gastrointestinal and intracranial hemorrhage. Careful examination of this patient's brain did not reveal any evidence of an amyloid angiopathy. The immunohistochemical results of brain tissue from the patients with sepsis are summarized in Fig. 2. Patient 1, a 56-year-old male control subject, died after having an RTA. There was diffuse staining of axons for βAPP restricted to the axonal hillock (Fig. 2a, *arrows*). More extensive axonal βAPP staining was seen in patient 2, a 55-year-old male patient with sepsis (Fig. 2b). There was beadlike swelling indicating axonal pathology. In addition, staining for nonphosphorylated NfH (SMI32)

Table 1 Brain tissue levels of total protein, glial fibrillary acidic protein, and neurofilament heavy chain in naive, sham-treated, and septic rats

Parameter	Naive group ($n = 4$)	Sham group ($n = 11$)	Septic group ($n = 14$)	p Value
Total protein levels, g/L	7.4 ± 2.6	8.7 ± 3.9	8.9 ± 2.9	>0.05
Brain tissue GFAP levels, ng/g total protein	0.34 ± 0.11	0.27 ± 0.19	0.29 ± 0.21	>0.05
Brain tissue NfH^{SMI35} levels, ng/g total protein	0.8 ± 0.6	2.6 ± 2.2	1.8 ± 1.7	>0.05

Abbreviations: GFAP Glial fibrillary acidic protein, *NfH* Neurofilament heavy chain, *Naive group* Controls (noninstrumented rats), *Sham group* Instrumented rats without injection of fecal slurry, *Septic group* Instrumented rats with injection of fecal slurry

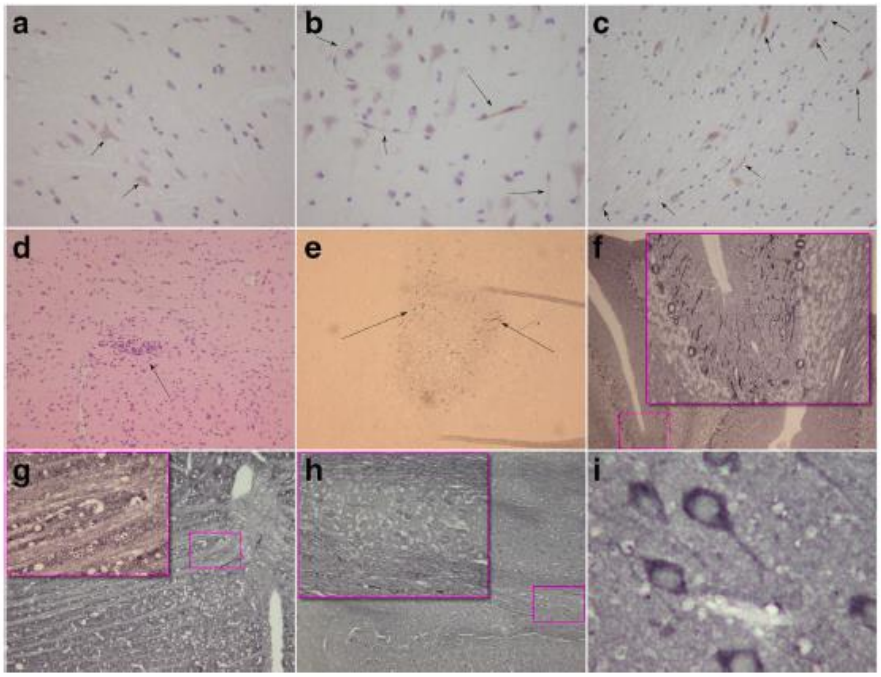

Fig. 1 Brain lesions seen in rat sepsis model. **a** Central brain white matter immunohistochemistry in sham-treated animals (controls) shows characteristic neuronal soma with restricted staining for β-amyloid precursor protein (βAPP) (*arrows*). **b** In septic animals, brain tissue from the same locations showed abnormal and more widespread axonal staining extending from the axonal hillock to the proximal axon (*arrows*). **c** Abnormal axonal βAPP staining follows white matter tracts (*arrows*). **d** There are also pockets of inflammatory and ischemic brain lesions seen in the rat sepsis model (H&E stain; *arrow*). **e** Staining of such lesions shows intense neuronal and axonal staining for βAPP (*arrows*). **f** Staining of lesions of sham-treated animals for β-tubulin in the magnification field is crisp and shows integrity of the neuroaxonal compartment (overview, *inset*; original magnification ×10). **g** Likewise, the integrity of white matter tracts in sham-treated animals can be seen (×10; *inset*, original magnification × 40). **h** There is severe structural disorganization of the β-tubulin network in white matter tracts of the septic animals. **i** The level of structural β-tubulin disorganization in the septic rat brain is best observed at greater magnification (original magnification × 40) of the neuroaxonal compartment from the same location as that taken from the sham model shown in (**f**)

demonstrated the presence of a large amount of axonal endbulbs, a sign of axonal degeneration (Fig. 2c). Next, scattered ischemic lesions were visible in patient 3, a 67-year-old female who died as a result of multiorgan failure due to sepsis (Fig. 2d). Amyloid plaques were found only in a 79-year-old female (Fig. 2e). Her medical history was not suggestive of cognitive impairment such as that seen with a neurodegenerative dementia; however, a formal neuropsychological assessment done before occurrence of sepsis was not available. Of note, the degree of diffuse deep white matter axonal injury was the most severe of all patients with sepsis (Fig. 2f).

Clinical presentation of human septic brain injury in vivo

Clinical presentation of SAE and mortality in patients with sepsis

The baseline characteristics of the septic cohort are summarized in Table 2. In 10 of the 13 patients, clinical signs of SAE were present at the onset of sepsis. Three patients developed septic shock perioperatively, requiring continued ventilation and sedation and thus precluding reliable cognitive assessments. Longitudinal CAM-ICU scoring was positive in 8 of the 13 patients. Three patients died within the 100-day follow-up period.

EEG findings in patients with sepsis

An EEG recording was done within a median of 2 days (range 0–4) after the onset of sepsis. At the time of recording, 7 of the 13 patients did not require sedation, and the remaining 6 had their sedation interrupted 30 minutes prior to undergoing EEG. EEG revealed encephalopathy of different extents in all patients (Table 3) Additional file 1.

MRI findings in patients with sepsis

Patients underwent MRI examination as soon as their clinical situation was deemed stable for in-house transfer. The median time from the onset of sepsis to brain MRI was 9 days (range 4–27). Reasons for delayed time to MRI were repeated surgical interventions in patients 1 and 11 and organizational reasons in patient 7. The MRI showed brain injury in 9 of 13 patients (Table 3, Fig. 3). The first pattern of WMH was punctiform ($n = 3$), patchy/confluent

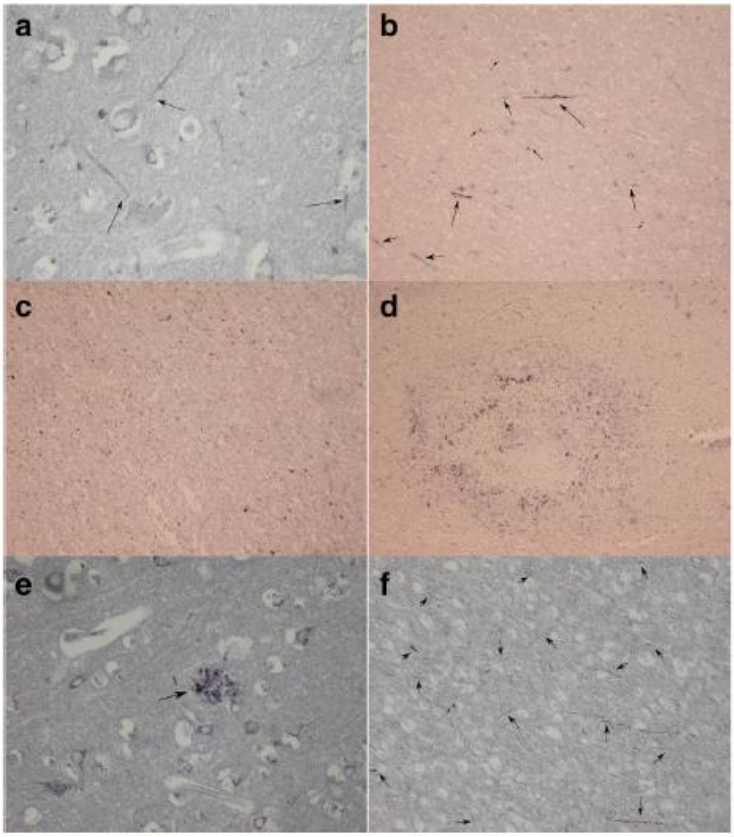

Fig. 2 Brain lesions seen in postmortem human tissue. **a** Patient 1 (control): Diffuse staining of axons (*arrows*), extending from the axonal hillock (β-amyloid precursor protein [βAPP]). **b** Extensive diffuse axonal injury is shown in patient 2 (sepsis). Staining for βAPP is not restricted to the axonal hillock but is seen throughout the white matter tracts. Multiple axonal endbulbs can also be seen (*small arrowheads*). **c** Disruption of the deep white matter axons and the presence of axonal end bulbs are widespread based on dephosphorylated neurofilament heavy chain (SMI32). **d** Patient 3: Small areas of ischemic lesions can be seen throughout the brain (βAPP). **e** Patient 4: One type of lesion not observed in the animal model is shown. Amyloid plaques (arrow) are present and scattered throughout the brain tissue (βAPP). **f** In this patient, diffuse deep white matter axonal damage (arrows) is the most severe of this series (βAPP)

Table 2 Clinical data of 13 patients with sepsis

Patient/ sex	Age (years)	APACHE II/worst SOFA	Ventilation (days)	SAE at sepsis onset	Positive CAM-ICU during ICU stay	ICU stay (days)	Hospital stay (days)	Survival at day 100
1/F	63	20/18	72	Yes	Yes	72 (dead)	72 (dead)	No
2/F	82	29/15	12	Yes	Yes	22	30	Yes
3/M	73	42/15	20	n.a.	No	20	20	Yes
4/M	57	27/11	2	Yes	Yes	9	29	Yes
5/M	55	12/6	0	Yes	No	3	30	Yes
6/F	80	24/12	2	Yes	Yes	20 (dead)	20 (dead)	No
7/M	44	40/8	10	Yes	Yes	20	33	Yes
8/F	76	39/12	16	n.a.	Yes	16 (dead)	16 (dead)	No
9/F	74	23/13	9	Yes	No	21	31	Yes
10/M	75	37/10	2	Yes	Yes	4	26	Yes
11/F	54	48/11	8	n.a.	No	23	45	Yes
12/F	60	23/12	12	Yes	Yes	14	36	Yes
13/M	81	38/12	20	Yes	No	20	20	Yes

Abbreviations: APACHE II Acute Physiology And Chronic Health Evaluation II score at ICU admission, *CAM-ICU* Confusion Assessment Method in the Intensive Care Unit, *ICU* Intensive care unit, *n.a.* Not applicable (analgosedation), *SAE* Sepsis-associated encephalopathy, *SOFA* Sepsis-related Organ Failure Assessment

Table 3 Results of magnetic resonance imaging and electroencephalography

Patient	Days from sepsis onset to MRI	MRI white matter hyperintensities	MRI ischemic lesions	Days to EEG	EEG abnormalities
1	27	Diffuse	No	4	Theta activity
2	8	Diffuse	Yes	1	Theta activity
3	7	None	No	4	Theta activity
4	7	None	No	3	Theta activity
5	9	Patchy/confluent	No	0	Theta activity
6	9	Punctiform	Yes	2	Delta activity
7	12	None	No	4	Delta activity
8	4	Punctiform	No	1	Delta activity
9	10	Patchy/confluent	No	2	Delta activity
10	10	Diffuse	No	2	Theta activity
11	17	None	No	4	Theta activity
12	4	Punctiform	No	1	Delta activity
13	5	Patchy/confluent	Yes	2	Delta activity

EEG Electroencephalography, *MRI* Magnetic resonance imaging

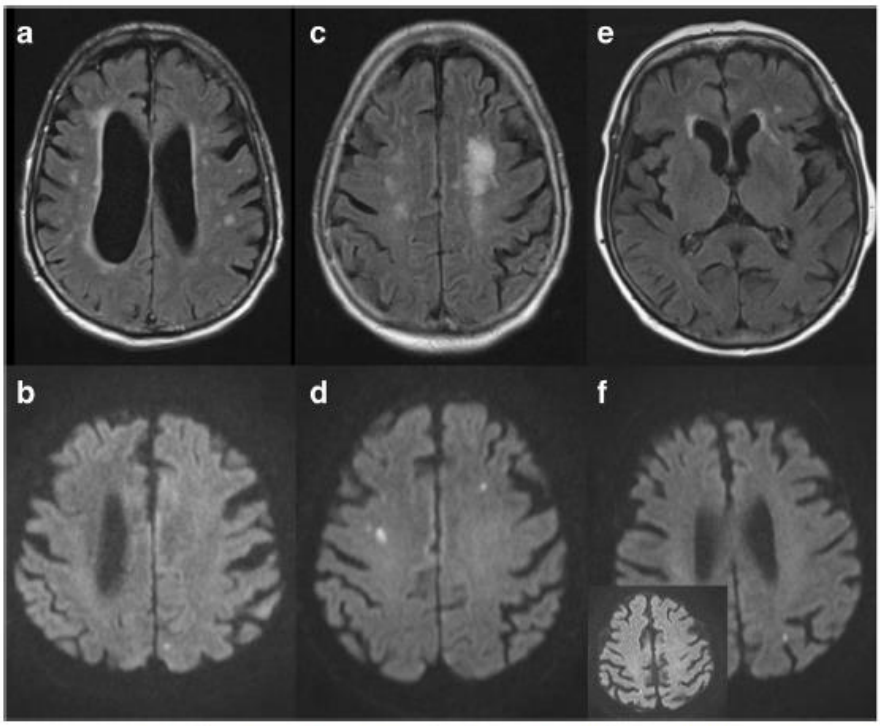

Fig. 3 Brain magnetic resonance imaging of three patients during septic shock. The images are fluid-attenuated inversion recovery (FLAIR; **a**, **c**, and **e**) and echo planar imaging diffusion-weighted imaging (DWI; **b**, **d**, and **f**) scans. **a** and **b** An 81-year-old male patient with urosepsis. **a** Axial FLAIR image obtained on day 5 after the onset of septic shock shows punctiform and confluent white matter hyperintensities (WMH) in both paraventricular and paramedian regions (grade 2 leukoencephalopathy). **b** DWI study shows subacute ischemic lesion in the left occipital paramedian region. **c** and **d** An 80-year-old female patient with urosepsis. (**c**) Axial FLAIR image obtained 9 days after the onset of septic shock shows confluent WMH in the left periventricular region (grade 2 leukoencephalopathy). **d** DWI study shows bilateral ischemic lesions in the frontal region. **e** and **f** An 80-year-old female patient with urosepsis. **e** Axial FLAIR performed 8 days after onset of septic shock revealing a single punctiform WMH in the left periventricular region (grade 1 leukoencephalopathy). **f** DWI study shows punctiform ischemic lesions in the left occipital and parietal (*inset*) regions

($n = 3$), or diffuse ($n = 3$). The second pattern of WMH was ischemic ($n = 3$) (Table 3, Fig. 3).

Discussion

The present translational study was performed to demonstrate evidence of axonal injury in the septic brains of animals and humans. Previous animal studies offer some evidence for different pathologic mechanisms of septic brain injury and behavioral changes [8–10, 12, 14, 39]. The histologic findings in the rat brains in the present study suggest two major mechanisms of injury. By βAPP staining, both diffuse axonal injury (DAI) and ischemic lesions were visible in the septic rat brain but not in sham-treated animals. Furthermore, βAPP staining showed abnormal axons along the white matter tracts. Pockets of inflammatory and ischemic lesions were detected by H&E staining. Further evidence for brain lesions in terms of disorientation of white matter was seen in β-tubulin staining of the septic animals but not the sham-treated group.

Our postmortem histologic samples of human septic brains showed results comparable to those derived from animal studies. Both βAPP staining and immunohistology for nonphosphorylated NfH monoclonal antibodies (SMI32) indicated DAI. Amyloid plaques were found only in human septic brains with βAPP staining, but they were completely absent in the rat histology. In contrast, Schwalm et al. reported increased Aβ levels in experimental sepsis [14]. Sharshar et al. noted various cerebral pathologies in patients who died as a result of sepsis, including ischemic lesions and neuronal apoptosis [40, 41]. The results of our present study are therefore in line with these previous reports, although our neuropathologic findings point to ischemia and DAI as the main mechanisms of brain injury. Owing to a longer interval from death to brain sampling in the patients, the histologic detection of DAI could represent a neuropathologic artifact. The importance of amyloid plaques within the human but not the animal brain histology is uncertain. These plaques could have predated the fatal sepsis episodes. Currently, on one hand, it can only be speculated that patients with amyloid plaques have a higher risk of developing SAE. Amyloid plaques, on the other hand, could be the result of a severe inflammatory stimulus to the brain, resulting in neurodegeneration and SAE, as shown before [14].

A limitation of both the human postmortem and experimental histologic studies was that investigations were restricted to brain tissue. There was no assessment of spinal cord tissue. The longest and, for DAI, most vulnerable axons travel through the spinal cord white matter tracts. We have previously demonstrated spinal cord involvement in a postmortem study of patients who died following West Nile virus infection [42]. Therefore, this study falls short of a conclusive demonstration of the anatomical distribution of diffuse white matter tract axonal injury in sepsis. Another limitation in this context is the lack of quantitative neuropathology. A potential future study may need to consider to correlate histological neuron/axon count with tissue body fluid levels [43].

It would clearly be useful to diagnose brain injury in patients with sepsis as early as possible for neurologic prognostication [21, 44]. Compared with improvements within neuropathology to detect DAI, routinely available in vivo diagnostics still lack the precision to accurately diagnose brain injury [45, 46]. In all clinically assessable patients with sepsis in the present study, clinical signs of SAE were present at the onset of sepsis, and this is consistent with pathologic EEG findings, as reported before [37, 47]. Nevertheless, the value of results derived from EEG examinations in the ICU setting is still under debate [19]. There was no statistically significant correlation between EEG and MRI results. We found that delirium screening systems such as CAM-ICU could not reliably detect SAE at all study points, owing to a high proportion of patients requiring sedation and mechanical ventilation. A clinical bedside diagnosis of SAE is not routinely feasible and is a well-recognized shortcoming of clinical assessment in ICU patients [19, 20, 48]. Specific biomarkers for neuroaxonal injury could be helpful in diagnosing DAI or SAE in vivo [49]. Previous clinical studies have been focused on neuron-specific enolase and S100B to diagnose brain injury in sepsis, with heterogeneous results [23]. Elevated Nf levels as markers of axonal injury were detected in various neurological conditions and may be of future use in SAE [49, 50].

In line with previous reports [21, 22], cerebral MRI did indicate brain injury in the majority of our patients. MRI findings suggested different extents of acute brain injury. In addition to WMH, acute or subacute ischemic lesions were detected in some of our patients. Compared with diffusion tensor imaging (DTI), conventional MRI techniques underestimate the extent of DAI [51, 52]. We can only hypothesize about more extensive axonal injury in our patients because they were examined only by MRI. Consistent with our postmortem neuropathological findings, the in vivo MRI results confirmed axonal and ischemic brain injury in our patients. We assume the inflammatory pockets in the postmortem histology to be correlates to the punctiform WMH seen on MRI scans. Although patients with any preexisting central nervous system pathology were not included in our study, we cannot exclude that some patients might have had subclinical brain injury before study inclusion that was interpreted as newly detected WMH. However, the ischemic lesions seen on MRI scans were undoubtedly newly present and provide striking evidence for ischemic brain injury during sepsis.

Conclusions

Axonal and ischemic brain injuries were detected in septic rat and postmortem human brain neuropathology and appear to be important mechanisms underlying SAE. The MRI findings of ischemic lesions and WMH were the best correlates to neuropathology. For in vivo detection of axonal injury in SAE, Nf body fluid levels should be analyzed longitudinally. DTI could be an option for detecting DAI in vivo more appropriately and should be considered in further clinical studies.

Additional file

Additional file 1: EEG delta activity. (PNG 640 kb)

Abbreviations

Aβ: β-Amyloid peptide; APACHE II: Acute Physiology and Chronic Health Evaluation II; βAPP: β-Amyloid precursor protein; CAM-ICU: Confusion Assessment Method in the Intensive Care Unit; DAI: Diffuse axonal injury; DTI: Diffusion tensor imaging; DWI: Diffusion-weighted imaging; EDTA: Ethylenediaminetetraacetic acid; EEG: Electroencephalography; ELISA: Enzyme-linked immunosorbent assay; FLAIR: Fluid-attenuated inversion recovery; GFAP: Glial fibrillary acidic protein; MRI: Magnetic resonance imaging; Nf: Neurofilaments; NfH: Neurofilament heavy chain; RASS: Richmond Agitation-Sedation Scale; RTA: Road traffic accident; SAE: Sepsis-associated encephalopathy; SOFA: Sepsis-related Organ Failure Assessment; WMH: White matter hyperintensities

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JE, MSa, and AP conducted the study, performed the statistical analyses, and wrote the manuscript. GNS and MSi edited the manuscript and provided conceptual advice. LKB, VT, and MSi performed the animal experiments. MGr and FS performed all neuropathological observations. MW, SK, JH, and MGl longitudinally assessed the septic patient cohort and provided data for this analysis. TS, FC, and FG assessed the patients in the postmortem sepsis group and provided all data for this analysis. AG evaluated all MRI scans and provided the MRI data for this analysis. All authors contributed significantly to the scientific content of the manuscript, and all authors read and approved the final manuscript.

Authors' information

Not applicable.

Ethics approval and consent to participate

All experiments were performed according to the University College London Ethics Committee and Home Office (UK) guidelines under the 1986 Scientific Procedures Act. The study was approved by the local ethics board at Rostock University (A 2012-0058) and by the Comité Consultatif de Protection des Personnes se Prêtant à la Recherche Biomédicale de Saint Germain en Laye, France. All patients or their legal representatives signed written informed consent forms before study inclusion.

Consent for publication

Not applicable.

Competing interests

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Research article

Diagnostic value of NT-proCNP compared to NSE and S100B in cerebrospinal fluid and plasma of patients with sepsis-associated encephalopathy

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ABSTRACT

Sepsis-associated encephalopathy (SAE) has significant impact on the neurocognitive outcome of sepsis survivors. This study was conducted to analyze the amino-terminal propeptide of the C-type natriuretic peptide (NT-proCNP) as a biomarker for SAE in comparison to neuron-specific enolase (NSE) and S100B protein. Cerebrospinal fluid (CSF) and plasma samples from twelve septic patients with SAE and nine non-septic controls without encephalopathy were analyzed. The assessment of SAE comprised a neuropsychiatric examination, delirium screening using the confusion assessment method in the ICU (CAM-ICU) and magnetic resonance imaging (MRI) in all participants. NSE, S100B and NT-proCNP were measured in plasma at study days 1, 3 and 7 in sepsis patients, once in controls and once in the CSF of both groups. The long-term outcome was assessed using the validated Barthel index (BI). Plasma NT-proCNP levels were significantly higher in the sepsis cohort compared to controls with peak concentrations at study day 1 (10.1 ± 6.6 pmol/l vs. 3.3 ± 0.9 pmol/l; $p < 0.01$) and a decrease over time. Plasma NT-proCNP levels at day 7 correlated with NT-proCNP in CSF ($r = 0.700$, $p < 0.05$). A comparable decrease of significantly higher plasma S100B values in sepsis patients compared to controls was observed. Plasma NSE levels were not significantly different between both groups. CSF NT-proCNP levels just tended to be higher in sepsis patients compared to controls and tended to be higher in patients with septic brain lesions seen on MRI. In the sepsis cohort CSF NT-proCNP levels correlated with CSF Interleukin-6 (IL-6) levels ($r = 0.616$, $p < 0.05$) and systemic inflammation represented by high plasma procalcitonin (PCT) levels at day 3 ($r = 0.727$, $p < 0.05$).

The high peak concentration of plasma NT-proCNP in the early phase of sepsis might help to predict the emergence of SAE during the further course of disease. NT-proCNP in plasma might, in contrast to CSF, indicate neurological impairment in patients with SAE.

1. Introduction

Sepsis-associated encephalopathy (SAE) is a common clinical manifestation of brain dysfunction in sepsis patients and is associated with poor outcome of survivors [1–3]. Due to the high impact on the long-term neurocognitive function of sepsis survivors, SAE has relevant

Abbreviations: APACHE-II-Score, acute physiology and chronic health evaluation score; BI, Barthel index; CAM-ICU, confusion assessment method in the intensive care unit; CNP, c-type natriuretic peptide; CNS, central nervous system; DSM, diagnostic and statistical manual of mental disorders; DTI, diffusion tensor imaging; ECLIA, electrochemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; NSE, neuron-specific enolase; NT-proCNP, amino-terminal propeptide of the C-type natriuretic peptide; RASS, Richmond agitation and sedation scale; SAE, sepsis-associated encephalopathy; SOFA, sepsis-related organ failure assessment; WMH, white matter hyperintensities

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socioeconomic importance [1,4–6]. The early and accurate clinical diagnosis of SAE is frequently delayed in sedated and mechanically ventilated patients with septic shock [7,8]. Diagnostic instruments to diagnose SAE include the neuro-psychiatric examination using the Diagnostic and Statistical Manual of Mental Disorders (DSM) as a gold standard, delirium screening checklists include the Confusion Assessment Method in the ICU (CAM-ICU) and the Intensive Care Delirium Screening Checklist (ICDSC), brain imaging and electroencephalography (EEG) [7–16]. Body fluid biomarkers are further instruments to detect and monitor SAE [9,17–21]. In comparison to time consuming brain magnetic resonance (MRI) or diffusion tensor imaging (DTI) as well as a detailed neuropsychiatric examination, body fluid biomarkers are highly interesting for clinicians [22]. Biomarkers of brain injury could help elucidate pathophysiological processes during SAE and would be valuable to detect and monitor encephalopathy in patients who cannot be clinically assessed [22–25]. A majority of studies focused on neuron-specific enolase (NSE) and on the S100B protein, which is more specific for glial cell damage [17–20,26]. Their value for screening and monitoring of SAE is still controversial due to differing study results [3,17–20,27]. The biomarkers C-type natriuretic peptide (CNP) and its amino-terminal propeptide NT-proCNP belong to the family of natriuretic peptides, for which the highest concentrations have been measured in the central nervous system (CNS) [28,29]. CNP has been shown to exert different functional roles within the endothelial glycocalyx implicating a potential regulation of vascular permeability [30]. **NT-proCNP release can be triggered by inflammatory mediators like IL-1ß and TNFα [31,32]. This indicates a relevant role of systemic inflammation and sepsis for the secretion and regulation of NT-proCNP.** Furthermore, different mechanisms within the brain were observed including axonal development, memory impairment and synaptic plasticity [30,33–35]. **Increased NT-proCNP levels were detected in CNS inflammatory conditions [36]. Koziorowski et al. observed a correlation between TNFα, IL-10 and NT-proCNP levels in Parkinson's disease, which underlines the relevance of inflammatory stimulation on regional NT-proCNP release [36]. Furthermore, some evidence was given for NT-proCNP effecting specific brain structures [34,35]. Decker et al. demonstrated CNP to locally decrease hippocampal network oscillations suspected to impair memory and learning processes in rats [34]. Biro et al. observed NT-proCNP release causing an anxiolytic state in rats implicating a relevance of this peptide on behavioral changes [37]. This is most important as neuroanatomical studies in patients with SAE confirmed brain region-specific injury of hippocampus and frontal cortex areas [38]. Therefore, a pathophysiological relevance of NT-proCNP in SAE is suspected and favors this peptide to be investigated as a biomarker for SAE.**

Interestingly, central and peripheral forms of CNP seem to be regulated differently in CSF and plasma [29].

CNP transcripts and components of its signaling pathways were prominently found in the cerebral microvasculature and choroid plexus within the brain and spinal cord suggesting a direct secretion into the CSF which explains the higher concentrations in the CNS compared to the systemic circulation [29].

Up to date, few clinical studies have evaluated the prognostic value of NT-proCNP in patients with septic shock in relation to outcome [39–42]. The specific role of NT-proCNP as a biomarker for the assessment of SAE has not been studied before.

We conducted a proof of concept study to analyze the diagnostic role of NT-proCNP compared to NSE and S100B in CSF and plasma to detect and monitor SAE in septic shock patients.

2. Materials and methods

2.1. Design and ethical protocol

All clinical and diagnostic data of the present study were evaluated from a previous prospective, longitudinal observational sepsis study that was performed between 2012 and 2016 at the University medical center Rostock, Germany. The study was approved by the local ethics committee of Rostock University (identifier: A 2012-0058) and registered as a clinical trial (ClinicalTrials.gov NCT02442986). Written informed consent was obtained from all participants or their legal representatives prior to study inclusion. Furthermore, clinical and imaging data as well as plasma and CSF samples were derived from neurologic control patients at the department of neurology of the medical university of Vienna, Austria. The evaluation of patient data and body fluid samples in Vienna was approved by the Institutional Ethics Committee of the Medical University of Vienna (EK-NR 1005/2014).

2.2. Inclusion and exclusion criteria

Patients with SAE, aged ≥18 years, with severe sepsis or septic shock according to the sepsis criteria used at that time were included [43]. A diffuse brain dysfunction related to sepsis, but without evidence for a primary cerebral infection was defined as SAE [2]. Neurologic patients with the following inclusion criteria served as controls: absence of any neuro-inflammatory or neurodegenerative disease, absence of sepsis and encephalopathy, absence of brain lesions seen on MRI and CSF analysis without evidence for infection.

Exclusion criteria for all participants comprised any preexisting or current CNS disease including, e.g. ischemia, hemorrhage, tumor, infection or dementia. Additionally all patients with coagulopathy and active bleeding were excluded from this investigation.

2.3. Clinical and neurological assessment protocol of sepsis patients

All sepsis patients were assessed by an interdisciplinary team experienced in critical and neuro-critical care. The Acute Physiology and Chronic Health Evaluation (APACHE)-II score was used at the time of ICU admission and the Sepsis-related Organ Failure Assessment (SOFA) score during follow-up to assess patients' severity of disease [44,45]. The neuro-psychiatric assessment of SAE was performed by an experienced neurologist (MW) and included a detailed medical history from all patients, if obtainable, or information from their next of kin. Furthermore, a standardized neurological examination status including a detailed status of brainstem nerve reflexes and function, deep tendon reflexes, sensoric and motoric function was achieved as described in detail before [14]. The level of consciousness was assessed on the Glasgow Come Scale and the Richmond Agitation and Sedation Scale (RASS) [46,47]. After study inclusion patients were systematically evaluated for their level of consciousness and for signs of SAE as confusion, agitation, hallucination or acute changes in mental status by using the validated CAM-ICU [12,48].

2.4. Brain magnetic resonance imaging (MRI)

Details on the standardized MRI protocol that was used to assess SAE in septic patients were described elsewhere [14]. The extent of white matter hyperintensities (WMH) in the septic brain was based on a previously validated scale and WMH were scored according to their number and size from grade 0 (no lesions) to grade 1 (punctiform), grade 2 (patchy or confluent) and to grade 3 (diffuse) [14,16,49]. Furthermore, a standardized MRI protocol was used to assess the brain of control subjects. All imaging results were evaluated by an experienced neuroradiologist (AG) who was unaware of the patient condition.

2.5. NSE, S100B and NT-proCNP measurement

All plasma and CSF samples were centrifuged immediately (2000 *g* for 10 min), aliquoted and stored at −80 °C until analysis. NSE and S100B concentrations from CSF and plasma samples were measured

using a commercially available electrochemiluminescence immunoassay (ECLIA, Immulite, Siemens Healthcare GmbH, Erlangen, Germany). Furthermore, IL-6 levels were measured with the same ECLIA in CSF and plasma from all septic patients. The measurement of NT-proCNP was performed using a commercially available enzyme-linked immunosorbent assay (ELISA, BI-20872, Biomedica Immunoassays, Biomedica Medizinprodukte, Vienna, Austria) with a detection limit of 0.2 pmol/l. We processed each analysis twice, reporting the mean of the two measures (coefficient of variation 2.7%).

2.6. Long-term follow-up for 100 days

Long-term follow-up for 100 days after study inclusion was assessed on a validated scale. The Barthel index (BI) was used to assess the patients' activities of daily living [50,51]. The data were generated using a standardized telephone interview protocol with the patients or their next of kin [52].

2.7. Statistic analysis

The Shapiro Wilk test was used to test for normally distributed values. The two-sided *T*-Test was performed to test normally distributed values and the non-parametric Mann-Whitney-*U*-Test for non-normally distributed values. Correlation analyses were performed using Pearson's R for normally distributed and Spearman's R for non-normally distributed values. The Bonferroni method was used for multiple correlations of the three biomarkers NSE, S100B and NT-proCNP. Statistical significance was indicated at $p < 0.05$. All statistical analyses were performed in SPSS (IBM SPSS Statistics, Version 22, Chicago, IL, USA).

3. Results

3.1. Patient demographics and long-term outcome

Results from a total of twelve septic patients (mean age 67.8 ± 12.1 years) and nine neurologic controls (mean age 34.8 ± 13.1 years) were available for the analysis. An overview about the demographic details of the participants is shown in Table 1.

Five of twelve septic patients died within the observation period of 100 days. The mean Barthel index (BI) of the survivors was (82.1 ± 25.3). None of the nine control subjects died within 100 days. The BI was 100 in all controls (Table 1).

3.2. Neuropsychiatric assessment and confusion assessment method in ICU of patients and controls

A standardized neuropsychiatric assessment was performed in all participants. All septic patients showed typical signs of SAE like confusion, agitation, hallucination or acute changes in mental status. The CAM-ICU screening detected SAE in eleven out of twelve septic patients. None of the control subjects showed any signs of encephalopathy or focal neurologic deficits detected by neuropsychiatric assessment.

3.3. Brain lesions seen on magnetic resonance imaging in patients and controls

None of the control subjects had evidence for brain lesions detected on MRI. In the sepsis cohort MRI reports were available from nine patients. Six of these patients showed WMH suggestive of septic brain lesions. Details on MRI results in septic patients are provided elsewhere [14].

3.4. NSE, S100B and NT-proCNP levels in plasma of patients and controls

Plasma NSE levels did not significantly differ over time between both groups (Fig. 1A). Compared to the control group S100B levels were significantly higher in sepsis patients and decreased from day 1 to day 7 (day 1 $p < 0.01$, day 3 and day 7 $p < 0.05$; Fig. 1B). Likewise, NT-proCNP levels in plasma reached their peak concentration at day 1 and decreased over time in the sepsis cohort compared to controls (day 1 and day 3 $p < 0.01$, day 7 $p < 0.05$; Fig. 1C).

Table 1
Demographic details of patients and controls.

Patient/ Study code	Diagnosis	Age/ Sex	APACHE-II/ Worst SOFA	BI/mRS before study	Day 100 survival	Day 100 BI
1/1	Septic shock	82/f	29/15	90/1	Yes	75
2/10	Septic shock	60/f	23/12	70/3	Yes	100
3/11	Severe Sepsis	75/m	37/10	100/0	Yes	65
4/12	Septic shock	63/f	20/18	100/0	No	Dead
5/13	Septic shock	55/m	12/6	100/0	Yes	100
6/15	Septic shock	73/m	42/15	100/0	Yes	100
7/19	Septic shock	76/f	39/12	100/0	No	Dead
8/3	Septic shock	55/f	39/14	75/3	No	Dead
9/55	Septic shock	79/f	22/6	100/0	No	Dead
10/6	Septic shock	80/f	24/12	95/1	No	Dead
11/8	Septic shock	44/m	40/8	95/1	Yes	100
12/9	Septic shock	72/f	9/4	35/4	Yes	35
13/A01	Pseudotumor cerebri	20/f	n.a.	100/0	Yes	100
14/A02	Pseudotumor cerebri	25/f	n.a.	100/0	Yes	100
15/A03	Pseudotumor cerebri	26/f	n.a.	100/0	Yes	100
16/A05	Pseudotumor cerebri	41/f	n.a.	100/0	Yes	100
17/L48	Dysesthesia	33/f	n.a.	100/0	Yes	100
18/L50	Vertigo	31/f	n.a.	100/0	Yes	100
19/L52	Acute hearing loss	65/m	n.a.	100/0	Yes	100
20/L54	Acute headache	37/f	n.a.	100/0	Yes	100
21/L56	Acute headache	35/f	n.a.	100/0	Yes	100

APHACHE-II = Acute physiology and chronic health evaluation at ICU admission; BI = Barthel index; ICU = Intensive care unit; mRS = Modified Rankin scale; SOFA = Sepsis-related organ failure assessment.

3.5. NSE, S100B and NT-proCNP levels in cerebrospinal fluid of patients and controls

CSF analysis was performed in all twelve septic patients with a mean time from sepsis onset to lumbar puncture of 3.6 ± 1.8 days. None of the patients showed signs of a cerebral infection (leukocytes in CSF < 5 Mpt/l).

NSE levels were higher in the sepsis group compared to controls (mean NSE 8.0 ± 6.0 ng/ml vs. 3.8 ± 2.2 ng/ml, $p < 0.05$; Fig. 2A). Mean NT-proCNP levels (sepsis 352.2 ± 163.8 vs. controls 284.0 ± 156.9 pmol/l, $p > 0.05$; Fig. 2C) and mean S100B levels (sepsis 0.57 ± 0.2 ng/ml vs. controls 0.66 ± 0.4 ng/ml, $p > 0.05$; Fig. 2B) were not significantly different between sepsis patients and controls (Fig. 1).

3.6. Value of NT-proCNP, NSE and S100B in patients with SAE

3.6.1. Inflammation and septic brain lesions seen on MRI in SAE

To evaluate a potential link between inflammation and septic brain injury, NT-proCNP levels were correlated with IL-6 levels in CSF and plasma. Elevated CSF NT-proCNP levels were correlating with CSF IL-6 levels ($r = 0.616$; $p < 0.05$), in contrast to NSE and S100B. This correlation was neither observed between CSF NT-proCNP and plasma IL-6 levels over time nor between plasma NT-proCNP and plasma IL-6 levels. The CSF NT-proCNP levels strongly correlated with plasma PCT levels at day 1 ($r = 0.709$; $p < 0.05$), day 3 ($r = 0.727$; $p < 0.05$) and marginally at day 7 ($r = 0.600$; $p = 0.05$). A weak correlation was also detected between plasma NT-proCNP and plasma PCT levels at day 1

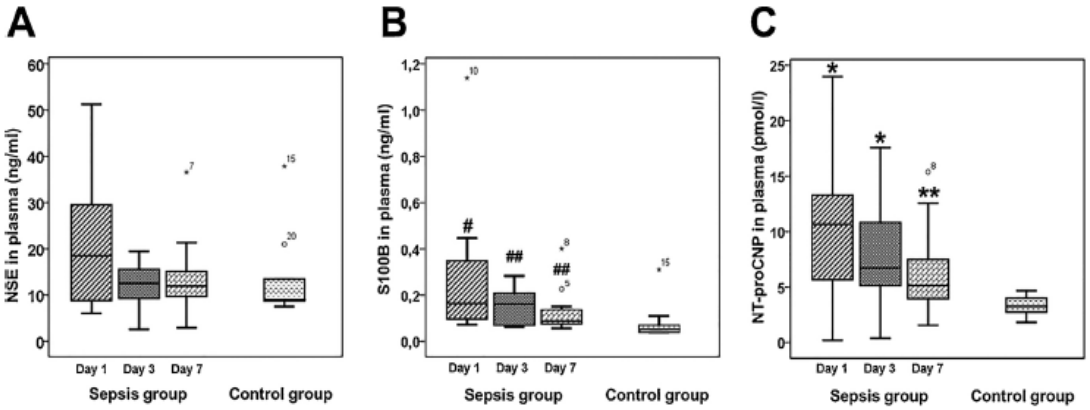

Fig. 1. NSE, S100B and NT-proCNP levels in plasma of sepsis patients and controls over time.
A: Plasma NSE levels in sepsis patients and controls ***B:*** Plasma S100B levels in sepsis patients and controls; # $p < 0.01$ between S100B at day 1 and controls; ## each $p < 0.05$ between S100B at day 3 and day 7 and controls). ***C:*** Plasma levels of NT-proCNP in sepsis patients and controls; each * $p < 0.01$ between NT-proCNP at day 1 and at day 3 and controls; ** $p < 0.05$ between NT-proCNP at day 7 and controls).

($r = 0.597$; $p < 0.05$), but not at day 3 or day 7. Neither CSF nor plasma NSE or S100B levels correlated with the extent of inflammation indicated by plasma PCT and IL-6 levels between day 1 and day 7. Furthermore, CSF NT-proCNP levels correlated with plasma NT-proCNP levels at day 7 ($r = 0.700$, $p < 0.05$, Fig. 3).

Mean CSF NT-proCNP levels just tended to be higher in patients with septic brain lesions seen on MRI compared to patients without brain lesions (389.2 ± 153.4 vs. 273.8 ± 269.8 pmol/l, $p > 0.05$; Fig. 4). No differences were detected between both groups for NSE and S100B levels in CSF.

3.6.2. Value of NSE, S100B and NT-proCNP levels to predict outcome in patients with SAE

Five out of twelve septic patients died during follow-up for 100 days. None of the three biomarker levels in CSF and plasma differed significantly between survivors and non-survivors at day 100.

The BI at day 100 was significantly lower in sepsis patients (mean BI 82.1 ± 25.3) compared to controls (mean BI 100 ± 0; $p < 0.05$). None of the three biomarkers, neither in CSF nor in plasma, correlated with the BI at day 100.

3.6.3. Correlation of age with NSE, S100B and NT-proCNP levels in CSF and plasma of sepsis patients and controls

None of the three biomarker levels in CSF and plasma correlated with age within the control group. In the sepsis cohort we observed no correlation between age and NSE and S100B levels in CSF and plasma. Furthermore, NT-proCNP levels in plasma did not correlate with patients' age

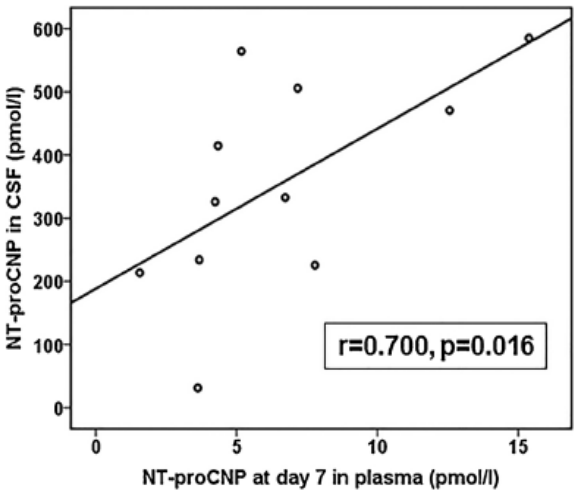

Fig. 3. Spearman's correlation analysis between CSF NT-proCNP and plasma NT-proCNP levels at day 7.

($r = 0.760$, $p < 0.01$).

4. Discussion

To the best of our knowledge this is the first observational study on the value of NT-proCNP levels in patients with SAE. The combined

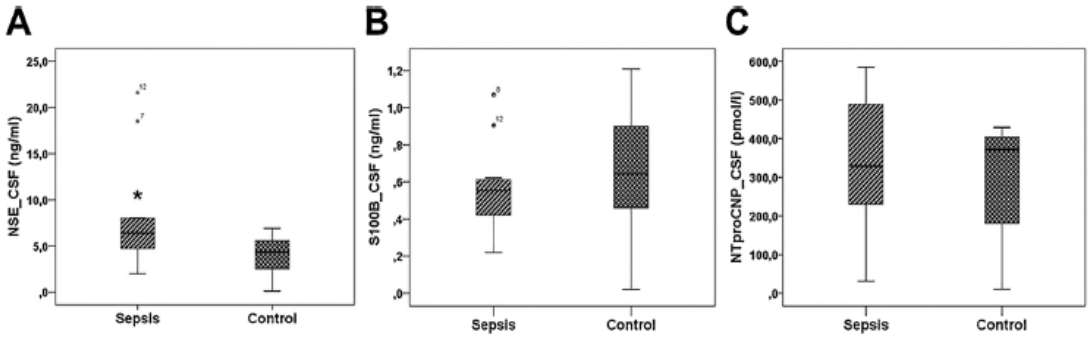

Fig. 2. NSE, S100B and NT-proCNP levels in CSF of sepsis patients and controls.
A: CSF NSE levels in sepsis patients and controls; * $p < 0.05$ between CSF NSE in sepsis patients and controls. ***B:*** CSF S100B levels in sepsis patients and controls. ***C:*** CSF NT-proCNP levels in sepsis patients and controls.

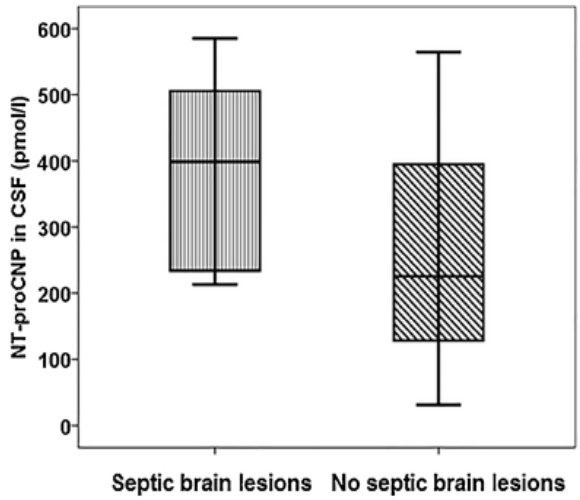

Fig. 4. NT-proCNP levels in CSF of sepsis patients with and without septic brain lesions seen on MRI.

analysis of NT-proCNP levels in CSF and the longitudinal measurement in plasma of septic patients delivers interesting new insights into the potential role of natriuretic peptides in neuro-inflammatory conditions. We conducted a proof of concept study between septic patients with SAE and neurologic patients without evidence for sepsis and encephalopathy to compare our findings with CSF and plasma levels of well-characterized controls. Furthermore, NSE and S100B levels in both body fluids were analyzed as these biomarkers were evaluated in the majority of preexisting SAE studies [17–20].

At the first glance biomarker findings in CSF of sepsis patients and controls within the present study showed no relevant differences between the groups. The CSF NSE levels did neither significantly increase in SAE patients nor in controls. Furthermore, no significant differences were observed for CSF S100B levels between both groups. These results are in line with previous controversial reports on the validity of NSE and S100B to evaluate SAE [9,17–21,53,54]. CSF NT-proCNP levels in the sepsis cohort just tended to be higher compared to controls but did not reach significance in the present study. We speculate that patients from our control group might not have been ideal candidates to present significant differences. These patients were not completely healthy but were best matched due to the availability of diagnostic results from neurological examination, brain imaging, CSF and plasma analysis. Therefore, especially NT-proCNP levels, suggesting neuro-inflammation, are suspected to be false high in our controls, but CSF was not available from healthy adults. Our results might on the other hand highlight the value of NT-proCNP measurement in CNS diseases. NT-proCNP even increases in controls with slight neurologic symptoms like headache or vertigo, thus supporting the role of NT-proCNP as a sensitive marker of neurologic impairment [40,41]. A major concern on CSF sampling in completely healthy controls is that lumbar puncture is an invasive procedure with potentially severe complications and is therefore not ethical [55–58]. A study from Tomasiuk et al. analyzed CSF NT-proCNP levels **in patients with meningitis** and reported median levels of 3.87 (IQR 2.3–6.1) pmol/L in healthy volunteers [41]. In comparison to these data, the CSF NT-proCNP levels in SAE patients of the present study (median NT-proCNP level 329.3, IQR 31.2–585.2 pmol/L) were significantly higher. This might underline the value of NT-proCNP measurement in CSF of SAE patients, although we were not able to show significant differences between sepsis patients and controls within this study. Nevertheless, compared to NSE and S100B, NT-proCNP seems to be much more sensitive to acute brain injury which might advocate this biomarker to be a better candidate for the detection of SAE.

Further evidence for the value of CSF NT-proCNP measurement in SAE patients was given with regard to septic brain lesions seen on MRI. CSF NT-proNCP levels tended to be higher in patients with septic brain lesions, although significance was not reached in our small patient population. The standard MRI examination, which was performed in the present study, might not have been sensitive enough to show the full extent of brain injury in SAE [59,60]. Future studies should focus on DTI to evaluate structural brain injury in sepsis [60,61].

The exact regulatory mechanisms of natriuretic peptides are still insufficiently understood [29,30,62]. Osterbur et al. detected an upregulation of the CNP production from the vascular endothelium of canine aortic cells after stimulation with lipopolysaccharide (LPS), tumor necrosis factor (TNF)-alpha and IL-1ß [32]. These factors are typically involved in the early systemic inflammatory response of the organism to an infection [63]. Elevated NT-proCNP levels were observed in CSF and plasma of septic patients, positively indicating an inflammatory stimulus to the production of natriuretic peptides and an increase of NT-proCNP in body fluids [40,41]. This is supported by our data as elevated CSF NT-proCNP levels correlated with IL-6 levels in CSF and plasma PCT levels, which was not observed for NSE and S100B values.

In contrast to the CSF analysis, evidence for the advantage of NT-proCNP measurement in SAE was given by plasma NT-proCNP levels measured in the present study. The high peak concentration of plasma NT-proCNP during the early phase of sepsis might be of value to predict the further development of SAE in sepsis patients. It additionally supports the potential link between inflammation and an upregulation of NT-proCNP [32,37]. This is also supported by the observed correlation between plasma NT-proCNP and plasma PCT levels at day 1. Interestingly, plasma NT-proCNP levels decreased during the further course of sepsis. We hypothesize that a declining inflammatory stimulus might result in less release of NT-proCNP during the course of sepsis. As natriuretic peptides are known to shed the endothelial glycocalyx and enhance vascular permeability, a typical phenomenon in sepsis, a shift of plasma NT-proCNP from the intra- to the extravascular compartment could additionally cause decreasing plasma levels [30]. Moreover, the successful therapy of sepsis and the decreasing inflammatory stimulus to the patients might result in decreasing NT-proCNP levels.

Despite all attempts to perform a standardized multimodal assessment of SAE in a matched cohort, several limitations of the present study have to be mentioned. The low number of study participants was mainly conditional due to the limited number of septic shock patients with available CSF findings. Otherwise, reports on CSF examinations in patients with early septic shock and signs of SAE are rare in literature, which is an advantage of the present study. The neurologic control patients were not ideally matched to present significant differences between the groups, but the availability of neuropsychiatric examinations, MRI reports, CSF and plasma samples to compare the biomarker findings between the groups were considerable advantages. The difference in years between the older sepsis patients and the younger controls was another factor that was not ideal in the present study. Otherwise age alone was not found to be relevant for our interpretation of biomarker results as no correlation was observed between plasma levels of any of the three biomarkers and age in both groups. Solely CSF levels of NT-proCNP correlated with age in the sepsis cohort, which might indicate towards a higher risk of brain injury in older patients during sepsis. Furthermore, the performance of MRI examinations in septic shock patients was not the best possible brain imaging technique. DTI would have been superior to conventional MRI techniques to display neuroaxonal injury in sepsis [60,61]. As DTI was not available in our hospital at that time we had to focus on conventional MRI. On the other hand MRI is more widely available and several studies reported on brain injuries in the course of septic shock, which was confirmed by the present study [9,14,16,64]. Future studies should include critically ill patients without sepsis to act as controls to compare clinical, imaging and plasma biomarker findings. **As it was not part of this proof of concept study, future studies should additionally focus on sepsis**

patients without SAE to evaluate the role of plasma NT-proCNP levels as a potential biomarker of SAE. However, CSF results would not be available in these patients as the performance of lumbar puncture without a clear clinical indication could not be ethically justified.

5. Conclusion

Due to the longitudinal development of the significantly higher plasma NT-proCNP levels over time in sepsis patients, plasma measurements of NT-proCNP might be of clinical value for the detection and monitoring of SAE. The measurement of plasma NT-proCNP levels might be superior to NSE and S100B levels in SAE. The correlation between plasma and CSF NT-proCNP levels suggests a link between neuro- and systemic inflammation in sepsis. Larger prospective clinical trials with well-matched patient cohorts are warranted.

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Conflict of interest

The authors declare that they have no conflicts of interest related to this manuscript.

Authors' contributions

Johannes Ehler: Designed the study, performed the statistics, wrote the manuscript; Thomas Saller: Carried out NT-proCNP measurements, wrote the manuscript; Matthias Wittstock: Performed all neuropsychiatric examinations of the sepsis patients; Paulus S Rommer: Performed all neuropsychiatric examinations of the control subjects; Daniel Chappell, Bernhard Zwissler, Gabriele Nöldge-Schomburg and Daniel A Reuter: critically revised the manuscript; Annette Grossmann: Evaluated all imaging results; Georg Richter: Technically supported the study, critically revised the manuscript; Martin Sauer: Designed the study and critically revised the manuscript. All authors approved the final version of the manuscript.

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RESEARCH ARTICLE

The prognostic value of neurofilament levels in patients with sepsis-associated encephalopathy – A prospective, pilot observational study

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Abstract

Sepsis-associated encephalopathy (SAE) contributes to mortality and neurocognitive impairment of sepsis patients. Neurofilament (Nf) light (NfL) and heavy (NfH) chain levels as biomarkers for neuroaxonal injury were not evaluated in cerebrospinal fluid (CSF) and plasma of patients with sepsis-associated encephalopathy (SAE) before. We conducted a prospective, pilot observational study including 20 patients with septic shock and five patients without sepsis serving as controls. The assessment of SAE comprised a neuropsychiatric examination, electroencephalography (EEG), magnetic resonance imaging (MRI) and delirium screening methods including the confusion assessment method for the ICU (CAM-ICU) and the intensive care delirium screening checklist (ICDSC). CSF Nf measurements in sepsis patients and longitudinal plasma Nf measurements in all participants were performed on days 1, 3 and 7 after study inclusion. Plasma NfL levels increased in sepsis patients over time ($p = 0.0063$) and remained stable in patients without sepsis. Plasma NfL values were significantly higher in patients with SAE ($p = 0.011$), significantly correlated with the severity of SAE represented by ICDSC values ($R = 0.534$, $p = 0.022$) and correlated with a poorer functional outcome after 100 days ($R = -0.535$, $p = 0.0003$). High levels of CSF Nf were measured in SAE patients. CSF NfL levels were higher in non-survivors ($p = 0.012$) compared with survivors and correlated with days until death ($R = -0.932$, $p<0.0001$) and

study). MPL is supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. HZ is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (not involved in this study), has served at advisory boards of Roche Diagnostics and Eli Lilly and has received travel support from Teva. The remaining authors (JE, MW, SK, MG, JH, AH, PSR, AG, TS, GR, GNS, MS) declare that there are no competing interests. *This does not alter our adherence to PLOS ONE policies on sharing data and materials.

functional outcome after 100 days (R = -0.749, p<0.0001). The present study showed for the first time that Nf levels provide complementary prognostic information in SAE patients indicating a higher chance of death and poorer functional/cognitive outcome in survivors.

Introduction

During the last decades the main focus of sepsis care has been directed towards short- and long-term survival of patients [1]. Consequently patient management has improved reducing the overall mortality [2]. An important contributor to mortality and long-term morbidity is sepsis-associated encephalopathy (SAE) [3–6]. SAE is defined as a diffuse brain dysfunction secondary to sepsis and without evidence of a primary CNS infection or encephalopathy due to other reasons [7]. The pathophysiology of SAE is still unexplained but risk factors are emerging [8–11]. Structural evidence for brain injury in sepsis comes from imaging and neuroanatomy studies [9–13]. Clinical assessment of SAE is hampered by the altered level of consciousness due to sedation and the need for mechanical ventilation [6,14]. Neuropsychiatric examination, electroencephalography (EEG), neuroimaging and laboratory tests permit to monitor SAE [15–17]. Diagnostic accuracy especially of clinical examination and EEG monitoring, however remain low in more severe cases potentially confounded by the use of sedation [17]. Furthermore, the need for prolonged registration of EEG to detect abnormalities over time is not practicable in the ICU setting and previous studies showed no association between EEG and brain dysfunction detected by CAM-ICU [18]. In this context body fluid biomarkers may be of diagnostic value [6,19,20]. A common limitation to previous studies on SAE was that biomarkers investigated are not specific for the neuro-axonal compartment and results have been contradictory [21–26]. A more specific biomarker for neuro-axonal injury, the neurofilament proteins (Nf) can be accurately measured from the cerebrospinal fluid (CSF) and blood and consistently correlated with brain injury, disease severity and survival in a range of neurological diseases [9,27–32]. Nf proteins are an important part of the axonal cytoskeleton and represent an architectonic stable tube system [30]. They are classified as intermediate filaments of type IV [30]. As a consequence of axonal injury Nf are released into the extracellular fluid and can be measured by ELISA technique [30]. This is the first study on the value of neurofilament heavy (NfH) and neurofilament light chains (NfL) in cerebrospinal fluid (CSF) and plasma of patients with SAE. The aim is to evaluate the potential suitability of Nf as biomarkers to detect SAE, septic brain injury and to predict outcome in patients with sepsis.

Methods

Study design and ethical protocol

We conducted a prospective, longitudinal single-center exploratory study at three ICU at the university medical center Rostock, Germany. The patient recruitment period was between May 2012 and November 2016. All patients or their legal representatives signed a written informed consent form before study inclusion. The study was registered as a clinical trial (ClinicalTrials.gov: NCT02442986) and was approved by the local ethics board at Rostock University (A 2012–0058).

Inclusion criteria for participants were patients with an age $\geq$ 18 years and an inclusion within 24 hours after the beginning of severe sepsis or septic shock according to the sepsis criteria used at that time [33]. Exclusion criteria for all participants were evidence for any preexisting neuromuscular disease like diabetic, alcoholic polyneuropathy or inflammatory

neuropathies. Additionally, patients with a history of CNS diseases like dementia, ischemia or hemorrhage were excluded. Furthermore, coagulopathy with active bleeding, no informed consent by legal representatives, high-dose glucocorticosteroid treatment, preexisting renal replacement therapy and expected death within 12 hours were exclusion criteria.

Participants with an expected length of ICU stay of more than 48 hours but without sepsis and without brain dysfunction were included as controls. Except for MRI and lumbar puncture these control subjects had the same longitudinal assessment as sepsis patients.

Multimodal assessment protocol for sepsis-associated encephalopathy

Clinical assessment and long-term follow-up. Patients were longitudinally assessed for their time of ICU and hospital stay by an interdisciplinary team consisting of experienced neurologists and intensivists. Recommended severity of disease scales including the Acute Physiology and Chronic Health Evaluation II (APACHE-II) score at ICU admission and the Sepsis-related Organ Failure Assessment (SOFA) score at study days 1, 3, 7 and 28 were used [34–36]. All ICU patients were treated as recommended by international guidelines [2,33]. The length of ICU and hospital stay, days on the ventilator and 28- and 100-day survival were recorded from all participants. The Barthel index (BI) before hospital admittance and at day 100 after study inclusion was used to assess patients' activities of daily living and to evaluate patients' long-term functional outcome [37,38]. A standardized telephone interview with the patients or their legal representatives was conducted to ascertain the BI at day 100.

Neuropsychiatric assessment. SAE was defined as a diffuse brain dysfunction secondary to sepsis and without evidence of a primary CNS infection or encephalopathy due to other reasons [7].

All participants were assessed for clinical signs of brain dysfunction by neuropsychiatric examination within one day after study inclusion by an experienced neurologist (MW). This included a detailed medical history from the patient or their legal representatives for early clinical signs of brain dysfunction like confusion, agitation or reduced level of consciousness [39–41]. Furthermore, the evaluation of EEG recordings and the evaluation of CSF results completed the neuropsychiatric assessment performed by the neurologist. A standardized neurologic examination included brainstem function. Based on this neuropsychiatric assessment the diagnosis of SAE was made.

Confusion assessment method for the ICU (CAM-ICU) and Intensive Care Delirium Screening Checklists (ICDSC). The patients' level of consciousness was assessed by physicians experienced in intensive (MS, JE) and neuro-intensive care (JE) on day 1, 3, 7 and 28 using the Glasgow Coma Scale (GCS) and the Richmond Agitation and Sedation Scale (RASS) [36,42]. The longitudinal assessment of brain dysfunction was performed using CAM-ICU and ICDSC as validated scales to detect signs of delirium [9,39–41]. According to recommendation CAM-ICU was only performed in patients with a RASS above -4. A patient was defined as CAM-ICU positive if CAM-ICU screening was positive at least at one time point of assessment.

Electroencephalography and magnetic resonance imaging. Within 72 hours after study inclusion all patients underwent EEG examinations. EEG recordings (ED 14; Madaus Schwarzer, Munich, Germany) were performed over a time of 30 mins and were assessed by an experienced neurologist (MW). Details on the methods of EEG recording and the classification of EEG findings using the Young scale were described elsewhere [9,43]. Furthermore, a standardized MRI protocol was used in septic patients to detect brain injury in SAE as described in detail before [9,13]. In brief, septic shock patients were examined by MRI (1.5-T magnet system MAGNETOM Avanto, Siemens Healthcare, Erlangen, Germany; 3.0-T mangnet system MAGNETOM Verio, Siemens Healthcare) as soon they were stable for in house transfer. The

extent of white matter hyperintensities (WMH), an imaging marker of septic brain damage, was assessed by a neuroradiologist (AG) according to a validated scale [13]. This 4-graded scale describes WMH in the septic brain according to their number and size. The scale comprises grade 0 (no lesions), grade 1 (punctiform lesions), grade 2 (patchy or confluent lesions) and grade 3 (diffuse lesions) [13].

Neurofilament proteins

CSF samples for Nf measurements were derived from patients with clinical evidence for SAE and were taken by lumbar puncture within 72 hours after study inclusion. This time frame was set to achieve a hemodynamic stabilization of septic shock patients before lumbar puncture. Plasma samples were taken at days 1, 3 and 7. Neurofilaments were measured using two validated in-house developed ELISA kits [32,44,45]. All samples were batch analyzed in duplicates. The mean intra-assay coefficient of variation in our study was 3.24%.

Statistical analysis

All statistical analyses were performed in SAS (version 9.4). Normality was tested graphically and using Shapiro–Wilk statistics. Gaussian data were compared using the T-Test and non–Gaussian data with the non-parametric Wilcoxon test. A two–way unbalanced ANOVA (general linear model, GLM) was used for comparing more than two independent variables. Weighted power calculations were performed for an alpha of 0.5. Correlation analyses were performed using Pearson's R for Gaussian and Spearman's R for non–Gaussian data. Multiple correlations were corrected by the Bonferroni method.

Results

Patient demographics

Twenty five critically ill ICU patients were prospectively included, 20 patients with sepsis and five patients without sepsis serving as controls (Fig 1). MRI reports of 13 included patients were previously published with a focus on neuroaxonal injury in sepsis (9). In the present study a total of 20 patients with severe sepsis or septic shock, mean (SD) age 66.7 (14.0) years, eight male and twelve female, and five matched ICU patients without sepsis, mean (SD) age 61.2 (24.7) years, three of them male and two female, were enrolled (Table 1).

The mean BI of the controls (100) was not significantly different from the mean (SD) BI of the sepsis cohort (92.3 (15.9), $p>0.05$).

Clinical assessment of sepsis-associated encephalopathy in sepsis and control patients

SAE was diagnosed in 18 of 20 sepsis patients by neuropsychiatric assessment. CAM-ICU screening was positive for brain dysfunction in 16 of 20 participants. None of the five control subjects showed clinical signs of brain dysfunction according to neuropsychiatric assessment, CAM-ICU or ICDSC screening. Sepsis patients presented significantly higher mean (SD) ICDSC values in comparison to the control group (ICDSC 3.3 (2.2) in sepsis vs. 0.8 (0.45) in controls, $p = 0.025$).

Electroencephalography and magnetic resonance imaging in sepsis and control patients

EEG examination was performed in 24 of 25 patients. EEG was not available in one patient due to technical problems. The grade of EEG abnormalities differed between sepsis and

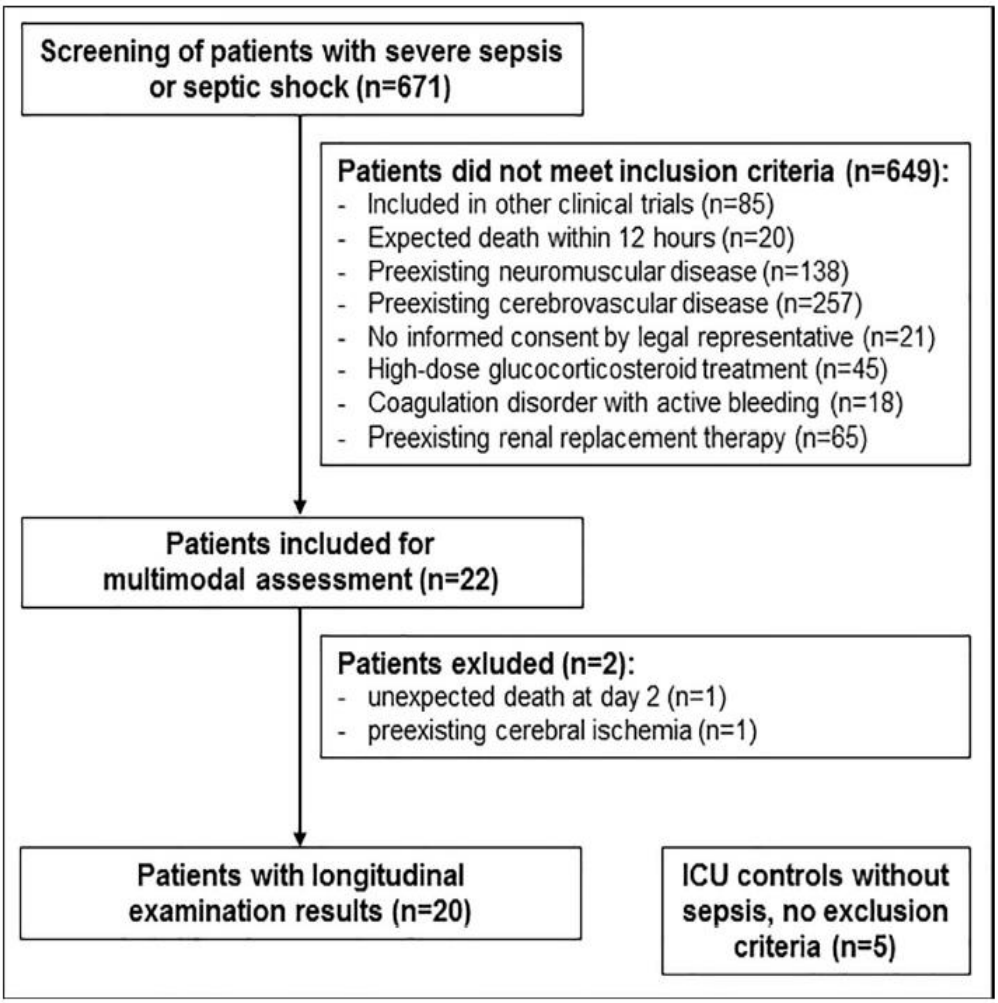

Fig 1. Study flow chart showing the prospective patient enrollment. ICU Intensive Care Unit.

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control patients (Table 2). None of the sepsis patients showed normal EEG activity (Table 2). In contrast, the majority of control patients showed normal alpha activity. Unexpectedly, triphasic waves were detected in one young female control patient (case #23) without evidence for SAE or any CNS disease. The patient refused cranial MRI examination which prevented further clarification.

WMH were detected in nine out of 13 sepsis patients with available MRI examinations. Additionally, subacute ischemic lesions were detected in three sepsis patients respectively. All EEG and MRI data are summarized in Table 2.

Long-term outcome after 100 days

Seven of 25 patients died within 100 days after study inclusion. Six non-survivors belonged to the sepsis group and one patient to the control group. The mean (SD) BI of sepsis survivors was lower than the mean (SD) BI of survivors of the control group (78.21 (29.7 vs. 95.0 (10.0), $p>0.05$).

Table 1. Patient characteristics of 25 study participants.

Patient	ICU cohort	Age/ Gender	BI before ICU	Sepsis condition	Sepsis focus	APACHE-II/ Worst SOFA	Ventilation (days)
1	Sepsis	63/F	100	Shock	Abdomen	20/18	72
2	Sepsis	82/F	90	Shock	Urogenital	29/15	12
3	Sepsis	85/F	95	Severe Sepsis	Abdomen	14/16	0
4	Sepsis	73/M	100	Shock	Abdomen	42/15	20
5	Sepsis	57/M	100	Shock	Abdomen	27/11	2
6	Sepsis	55/M	100	Shock	Abdomen	12/6	0
7	Sepsis	80/F	95	Shock	Urogenital	24/12	2
8	Sepsis	64/M	100	Shock	Soft tissue	11/14	27
9	Sepsis	72/F	35	Shock	Urogenital	9/4	0
10	Sepsis	44/M	95	Shock	Abdomen	40/8	10
11	Sepsis	76/F	100	Shock	Pulmo	39/12	16
12	Sepsis	74/F	90	Shock	Pulmo	23/13	9
13	Sepsis	72/F	100	Shock	Urogenital	38/10	0
14	Sepsis	75/M	100	Severe Sepsis	Urogenital	37/10	2
15	Sepsis	79/F	100	Shock	Soft tissue	22/6	1
16	Sepsis	32/M	100	Shock	Abdomen	19/9	4
17	Sepsis	54/F	100	Shock	Soft tissue	48/11	8
18	Sepsis	55/F	75	Shock	Soft tissue	39/14	6
19	Sepsis	60/F	70	Shock	Soft tissue	23/12	12
20	Sepsis	81/M	100	Shock	Urogenital	38/12	20
21	Control	74/M	100	None	n.a.	23/3	1
22	Control	63/M	100	None	n.a.	9/5	0
23	Control	18/F	100	None	n.a.	14/5	1
24	Control	74/M	100	SIRS	n.a.	17/5	0
25	Control	77/F	100	SIRS	n.a.	22/7	1

APACHE-II Acute physiology and chronic health evaluation score, *BI* Barthel index (activities of daily living), *F* Female, *ICU* Intensive care unit, *M* Male, *n.a.* Not applicable, *SIRS* Systemic inflammatory response syndrome, *SOFA* Sepsis-related organ failure assessment score.

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Plasma neurofilament levels in sepsis and control patients

Plasma NfL and NfH values were compared between 20 sepsis and five control patients. Significant differences were present for NfL when comparing sepsis and control patients over time. The mean NfL values at study day 1 were not statistically different between the groups,but over time NfL plasma values of sepsis patients were significantly higher in comparison to

Table 2. Electroencephalography and magnetic resonance imaging results from 25 study participants.

Patient cohort		EEG findings				MRI findings	
	Normal activity	Theta waves	Delta waves	Triphasic waves	Burst-suppression pattern	WMH present	Ischemic lesions present
SAE[a]	0/18	10/18	8/18	0/18	0/18	9/11	3/11
No SAE	1/2	1/2	0/2	0/2	0/2	0/2	0/2
Control[b]	3/4	0/4	0/4	1/4	0/4	n.a.	n.a.

EEG Electroencephalography, *MRI* Magnetic resonance imaging, *n.a.* Not applicable, *WMH* White matter hyperintensities, *SAE* Sepsis-associated encephalopathy.

[a] MRI reports available from 11/18 SAE patients

[b] EEG reports available from 4/5 controls.

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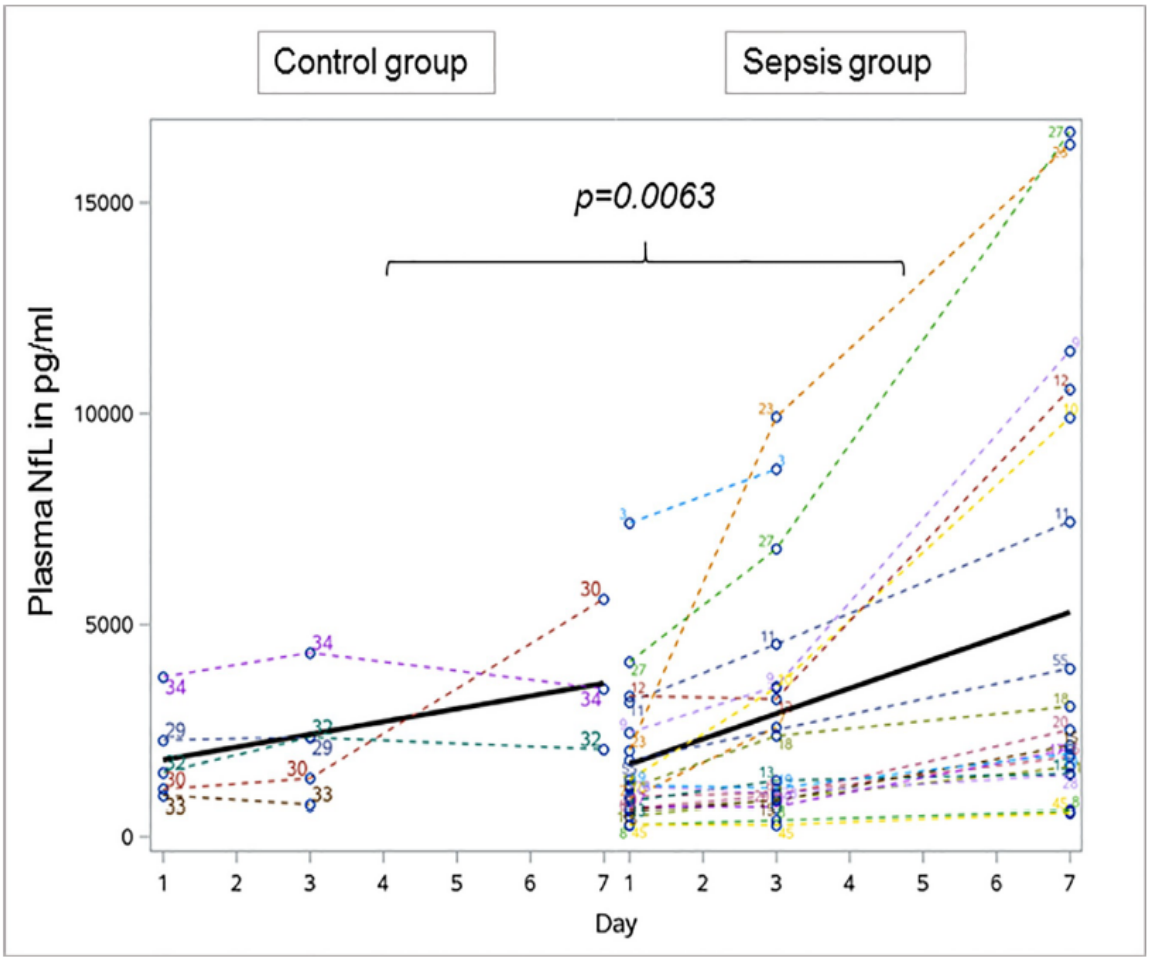

Fig 2. Longitudinal profile of neurofilament light chains over time in 20 sepsis and five control patients. The NfL levels significantly increased in sepsis patients over time and remained stable in controls. Bold line indicates the development of mean plasma neurofilament levels over time. NfL Neurofilament light chains.

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controls (GLM, p = 0.0063, Fig 2). Within the sepsis group plasma NfL levels significantly increased from day 1, mean (SD) NfL 1723.4 (1711.5) pg/mL to day 7, mean (SD) 5309.6 (5373.9) pg/mL ($p<0.001$) which was not observed in the control group (day 1, mean (SD) NfL 1905.2 (1151.9) pg/mL vs. day 7, mean (SD) NfL 3701.3 (1794.8) pg/mL, $p>0.05$).

Plasma NfH values were not significantly different between sepsis and control patients at day 1. Within the sepsis group a significant increase was observed from day 1, mean (SD) NfH 17.6 (41.5) ng/mL to day 7, mean (SD) NfH 163.4 (596.0) ng/mL (p = 0.043). This difference was not present in the control group (day 1, mean (SD) NfH 100.3 (221.4) ng/mL vs. day 7, mean (SD) NfH 519.9 (666.9) ng/mL, $p>0.05$).

An overview about the different development of NfL and NfH levels over time is given in Table 3.

Power calculations on these data indicate that a group size of n = 134 for plasma NfL, n = 126 for plasma NfH is needed to reach a power of 80% for separating sepsis from controls.

Plasma neurofilament levels in patients with sepsis-associated encephalopathy

Nf levels of 16 CAM-ICU positive patients were compared to four CAM-ICU negative patients. Mean (SD) NfL levels in CAM-ICU negative patients increased from 808.8 (245.2) pg/ml at day 1 to 1762.8 (370.5) pg/ml at day 7. Mean (SD) NfL levels in CAM-ICU positive patients significantly increased from 1952.0 (1849.2) pg/ml at day 1 to 6323.0 (5723.3) pg/ml at day 7 ($p = 0.001$). This increase over time was significantly stronger in CAM-ICU positive compared to CAM-ICU negative patients (GLM, $p = 0.011$, Fig 3). Next we corrected for missing samples using mixed models which confirmed the above finding ($p = 0.0007$). Peak concentrations of plasma NfL correlated with higher ICDSC values in sepsis patients ($R = 0.534$, $p = 0.022$). Mean (SD) NfH levels in CAM-ICU positive patients were higher at study day 1 (NfH 22.0 (45.6) ng/ml) compared to CAM-ICU negative patients (mean NfH 0.0 ng/ml) and further increased in CAM-ICU positive patients to a mean value of 211.3 (673.7) ng/ml at day 7, which was not observed in CAM-ICU negative patients (mean NfH 0.0 ng/ml). No significant group difference was observed for the development of NfH levels over time (Table 4).

Power calculations on these data indicate that for plasma NfL a group size of $n = 10$ is required on day one and of $n = 14$ on day seven to to reach a power of 80% for separating CAM-ICU positive from CAM-ICU negative patients.

MRI results were available from 13 sepsis patients. Septic brain injury, represented by different extents of WMH was detected in nine and not detected in four patients. Further details on MRI results are provided elsewhere (9). Patients with evidence for WMH tended to have higher plasma NfL values (mean (SD) NfL levels at day 1: 1405.0 (1063.5) pg/ml; at day 3: 2110.1 (1373.7) pg/ml; day 7: 4658.9 (3959.0) pg/ml) compared to patients without WMH (mean (SD) NfL at day 1: 665.8 (343.6) pg/ml, $p>0.05$; day 3: 1058.0 (882.1) pg/ml, $p = 0.045$; day 7: 1953.3 (1011.4) pg/ml, $p>0.05$) which correlated with the extent of lesions on MRI (Fig 4). In comparison to patients without WMH a significant increase of plasma NfL levels was detected in patients with WMH between day 1 and day 7 ($p = 0.012$).

Mean (SD) NfH levels slightly increased in patients with WMH from 22.9 (53.8) ng/ml at day 1 to 355.3 (887.0) ng/ml at day 7 ($p>0.05$) which was completely different to patients without WMH who did not show an increase of NfH levels over time (NfH 0 ng/ml at all three time points of measurement).

Table 3. Plasma neurofilament levels in sepsis patients and controls.

Neurofilament Light (NfL)	Sepsis group Mean (SD), pg/mL	Patients, No. (%) (n = 20)	Control group Mean (SD), pg/mL	Patients, No (%) (n = 5)	*p Value*[a]
Day 1	1723.4 (1711.5)	20 (100)	1905.2 (1151.9)	5 (100)	p>0.05
Day 3	2753.1 (2774.5)	20 (100)	2208.0 (1363.5)	5 (100)	p>0.05
Day 7	5309.6 (5373.9)	18 (90)	3701.3 (1794.8)	3 (60)	p>0.05
p value day 1 vs. day 7[b]	p<0.001		p>0.05		
Neurofilament Heavy (NfH)	Sepsis group Mean (SD), ng/mL	Patients, No. (%) (n = 20)	Control group Mean (SD), ng/mL	Patients, No (%) (n = 5)	*p Value*[a]
Day 1	17.6 (41.5)	20 (100)	100.3 (221.4)	5 (100)	p>0.05
Day 3	18.9 (63.2)	20 (100)	163.1 (350.2)	5 (100)	p>0.05
Day 7	164.3 (596.0)	18 (90)	519.9 (666.9)	3 (60)	p = 0.016
p value day 1 vs. day 7[b]	p = 0.043		p>0.05		

No, Number; SD, Standard deviation.

[a] p values calculated by comparing sepsis patients and controls.

[b] p values calculated by comparing neurofilament levels day 1 vs. day 7 within each study group.

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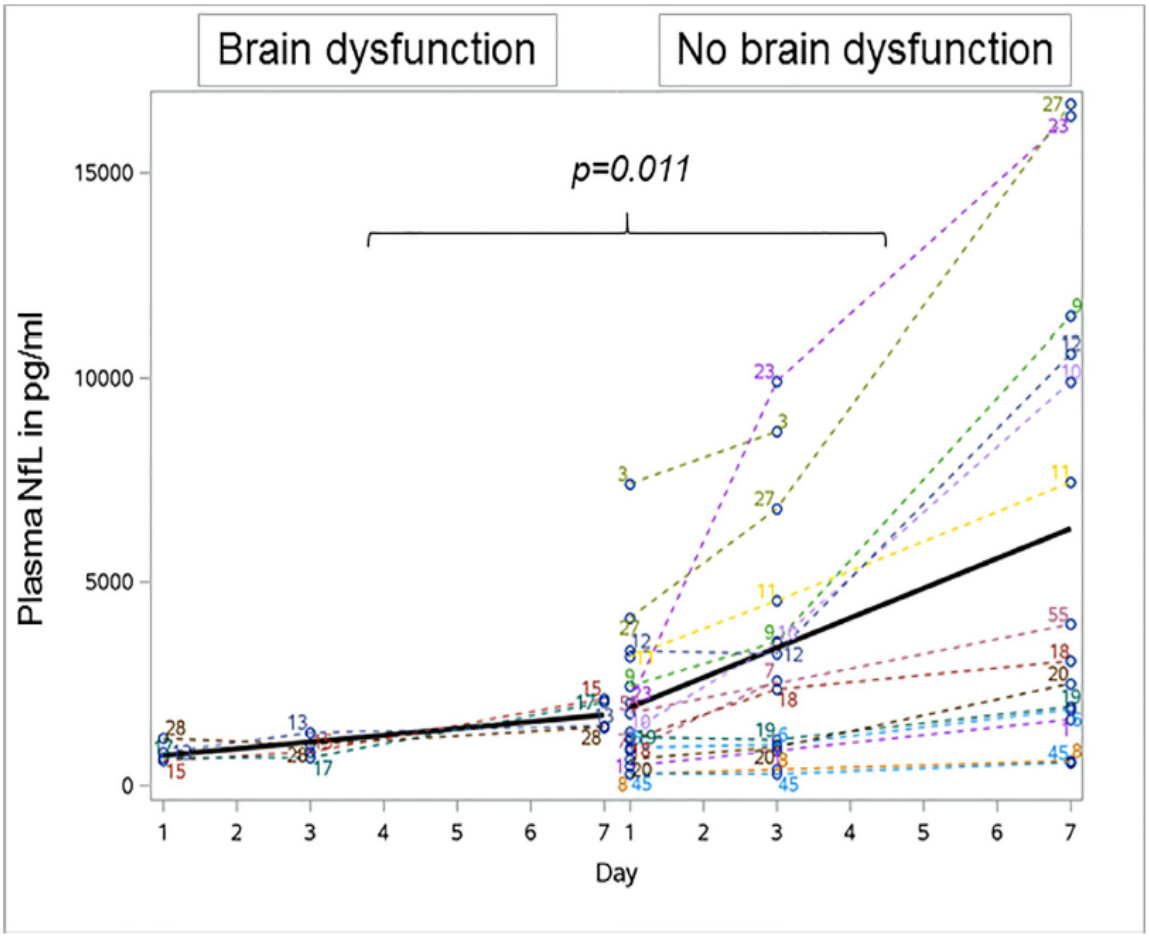

Fig 3. Longitudinal profile of plasma neurofilament light chain levels in 16 sepsis patients with brain dysfunction and four patients without brain dysfunction. NfL levels significantly increased in patients with brain dysfunction over time which was not observed in patients without brain dysfunction. Bold line indicates the development of mean plasma neurofilament levels over time. NfL Neurofilament light.

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Plasma neurofilament levels, 28-day and 100-day mortality in sepsis. Plasma Nf levels of four non-survivors at day 28 were compared to 16 survivors. Mean (SD) NfL levels in non-survivors increased from 2880.5 (3048.4) pg/ml at day 1 to 6724.7 (8367.0) pg/ml at day 7 ($p>0.05$). In survivors mean (SD) NfL levels at day 1 increased from 1434.1 (1185.6) pg/ml to 5026.6 (4954.9) pg/ml ($p = 0.001$).

Mean (SD) NfH levels in non-survivors at day 1 increased from 62.0 (77.6) ng/ml to 847.8 (1461.8) ng/ml ($p>0.05$) compared to survivors with a mean (SD) NfH level of 6.5 (18.0) ng/ml at day 1 and an increase to 27.6 (78.6) ng/ml ($p>0.05$).

Six non-survivors at day 100 were compared to 14 survivors. Mean (SD) NfL levels of the non-survivors significantly increased from 2765.3 (2417.0) pg/ml at day 1 to 6940.6 (6370.6) pg/ml at day 7 ($p = 0.043$). In survivors mean (SD) NfL levels increased from 1276.8 (1148.3) pg/ml at day 1 to 4682.3 (5084.2) pg/ml at day 7 ($p = 0.001$).

Mean (SD) NfH levels of non-survivors increased from 58.7 (60.5) ng/ml at day 1 to 586.2 (1096.2) ng/ml at day 7 ($p>0.05$). In survivors mean NfH levels increased from 0 ng/ml at day 1 to 2.1 (7.5) ng/ml at day 7 ($p>0.05$).

Table 4. Plasma neurofilament levels in patients with and without brain dysfunction detect by the Confusion assessment method for the ICU.

Neurofilament Light (NfL)	Brain dysfunction Mean (SD), pg/mL	Patients, No. (%) (n = 16)	No brain dysfunction Mean (SD), pg/mL	Patients, No (%) (n = 4)	*p Value*[a]
Day 1	1952.0 (1849.2)	16 (100)	808.8 (245.2)	4 (100)	p>0.05
Day 3	3205.0 (2940.7)	16 (100)	945.5 (265.3)	4 (100)	p>0.05
Day 7	6323.0 (5723.3)	14 (87.5)	1762.8 (370.5)	4 (100)	p>0.05
p value day 1 vs. day 7[b]	p = 0.001		p>0.05		
Neurofilament Heavy (NfH)	**Brain dysfunction Mean (SD), ng/mL**	**Patients, No. (%) (n = 16)**	**No brain dysfunction Mean (SD), ng/mL**	**Patients, No (%) (n = 4)**	***p Value***[a]
Day 1	22.0 (45.6)	16 (100)	0	4 (100)	p>0.05
Day 3	23.7 (70.3)	16 (100)	0	4 (100)	p>0.05
Day 7	211.3 (673.7)	14 (87.5)	0	4 (100)	p>0.05
p value day 1 vs. day 7[b]	p = 0.043		p>0.05		

No, Number; SD, Standard deviation.

[a] p values calculated by comparing sepsis patients and controls.

[b] p values calculated by comparing neurofilament levels day 1 vs. day 7 within each study group.

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Highly elevated cerebrospinal fluid neurofilament levels in patients with sepsis-associated encephalopathy. CSF samples were available from 12 of 20 sepsis patients. The mean (SD) time to CSF examination was 2.75 (2.1) days. Mean (SD) levels of NfH were 561.41 (1697.5) ng/ml and 21891.5 (49917.2) pg/ml for NfL (Table 5). In 8 of 20 patients lumbar puncture could not be performed due to disclaimer from legal representative after study inclusion (n = 4), unsuccessful puncture related to patient specific anatomical reasons (n = 3) and contraindication due to local soft tissue infection (n = 1).

Correlation of cerebrospinal fluid neurofilament levels with sepsis-associated encephalopathy, 28-day and 100-day mortality. Neuropsychiatric examination diagnosed SAE at the beginning of sepsis in all twelve patients with CSF analysis. Therefore, we were not able to compare CSF Nf levels between SAE positive and negative patients. CSF NfL levels were significantly higher in both 28- and 100-day non-survivors. In three non-survivors at day 28 the mean (SD) NfL level of 69,986 (94,939.1) pg/ml was significantly higher compared to a mean (SD) NfL level of 5860 (4027.5) pg/ml of nine survivors (p = 0.021). We measured significantly higher CSF NfL levels in five non-survivors at day 100 with a mean (SD) NfL level of 45,966.2 (74,840.8) pg/ml compared to seven survivors with a mean (SD) NfL level of 4695.3 (2465.3) pg/ml (p = 0.012, Fig 5). CSF NfH levels tended to be higher in septic non-survivors at day 28 (mean (SD) NfH level 2038.4 (3387.5) ng/ml) compared to survivors (mean (SD) NfH level of 69.0 (49.7) ng/ml; p>0.05). This trend was confirmed in non-survivors at day 100 with a mean (SD) NfH level of 1271.6 (2615.7) ng/ml compared to survivors with a mean (SD) NfH level of 54.1 (32.7) ng/ml (p>0.05).

Power calculations on these data indicate that a group size of n = 53 for CSF NfL, n = 72 for CSF NfH is needed to reach a power of 80% for separating survivors from non-survivors.

Correlation of cerebrospinal neurofilament levels with brain pathology seen on magnetic resonance imaging. Seven patients with WMH seen on MRI showed significantly higher CSF NfL levels, mean (SD) 8217.3 (6139.6) pg/ml, compared to two patients without WMH, mean (SD) CSF NfL 2958.0 (69.3) pg/ml (p = 0.017). No significant difference in CSF NfH levels was observed between patients with WMH (mean (SD) CSF NfH level 69.2 (23.8) ng/ml) and without WMH seen on MRI (mean (SD) CSF NfH level 35.5 (50.2) ng/ml, p>0.05).

Correlation of cerebrospinal fluid and plasma neurofilament levels with long-term functional outcome. We observed a negative correlation between CSF NfH levels and BI

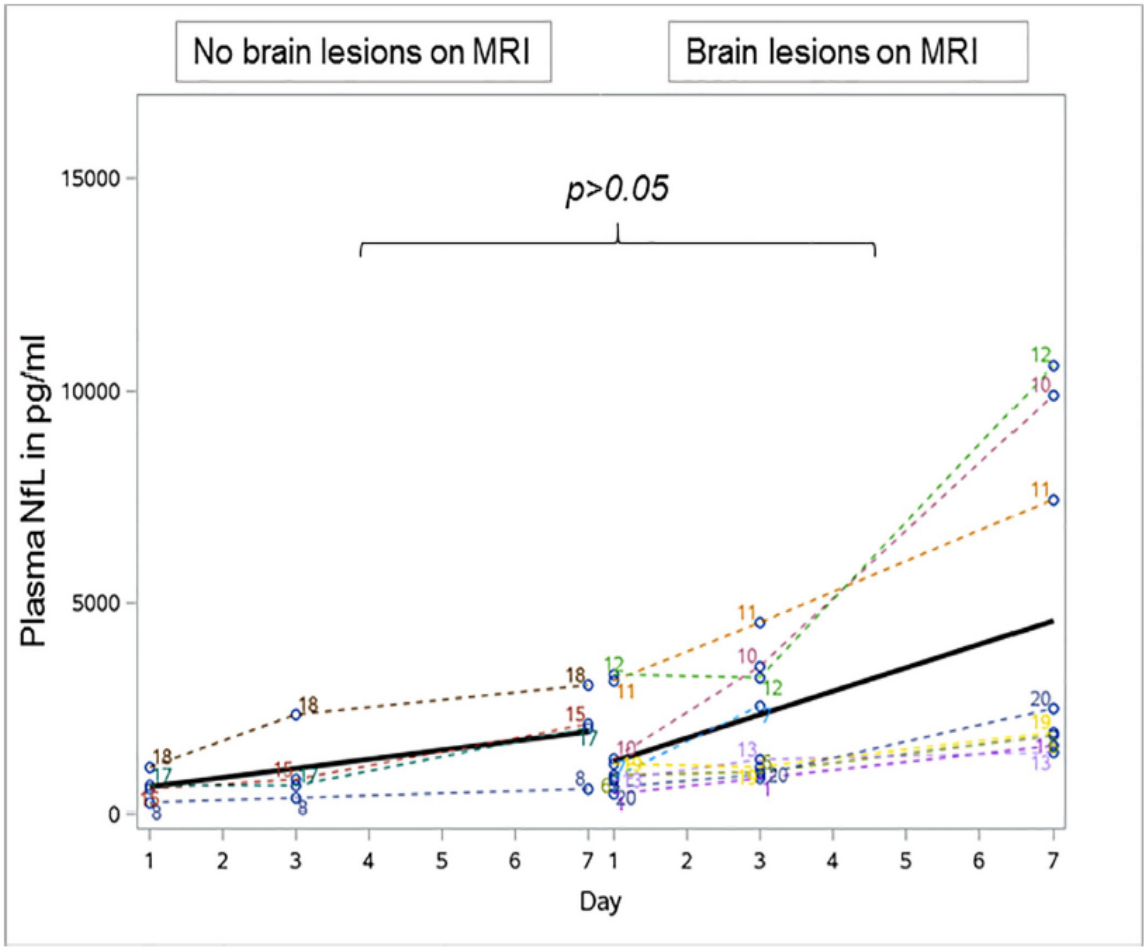

Fig 4. Development of plasma neurofilament light chain levels in patients with no brain lesions and brain lesions seen on magnetic resonance imaging in sepsis. No difference in the plasma NfL increase over time between septic patients with and without brain lesions seen on MRI. Bold line indicates the development of mean plasma neurofilament levels over time. NfL Neurofilament light chain, MRI Magnetic resonance imaging.

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before sepsis. A lower BI before hospital admittance was associated with higher CSF NfH levels (R = -0.490, p = 0.0028), which was neither present for CSF NfL nor for both plasma Nf levels. Higher CSF NfL (R = -0.749, p<0.0001) and higher plasma NfL levels (R = -0.535, p = 0.0003) correlated with a lower BI at day 100 representing a poorer clinical outcome of these patients. Additionally, a link to patient outcome was observed by correlating CSF NfH and NfL as well as plasma NfH values with the time to death in non-survivors. Higher Nf levels were associated with shorter survival of patients (Table 6).

Discussion

This prospective longitudinal exploratory study was conducted to evaluate the prognostic value of Nf levels in patients with SAE. Nf levels are known to be increased in several disorders with neuropsychiatric symptoms [27,31,46]. The potential value for SAE has not yet been investigated. Preexisting studies on SAE analyzed non-specific biomarkers like interleukin-6, neuron-specific enolase (NSE) or S100B protein [19, 21–23,47,48,49]. The results were found

Table 5. Neurofilament levels in cerebrospinal fluid of twelve sepsis patients.

Patient/ Study code	Study days to LP	Cell count in Mpt/l (Ref <5 Mpt/l)	Protein in mg/l (Ref 150–450 mg/l)	CSF NfH in ng/ml	CSF NfL in pg/ml
1	8	1	324	65.0	4908
2	2	1	561	87.2	9425
4	3	1	353	0	2909
6	2	3	302	25.2	2166
7	2	3	245	85.5	9822
9	2	1	209	53.9	4864
10	3	2	453	71.0	3007
11	1	3	326	79.9	20704
14	1	2	373	51.7	5961
15	3	3	282	177.5	14965
18	2	1	151	5949.9	179432
19	5	1	232	89.8	4535

LP Lumbar puncture, *Mpt/l* Megaparticels/liter, *NfH* Neurofilament heavy chain, *NfL* Neurofilament light chain, *Ref* Reference range.

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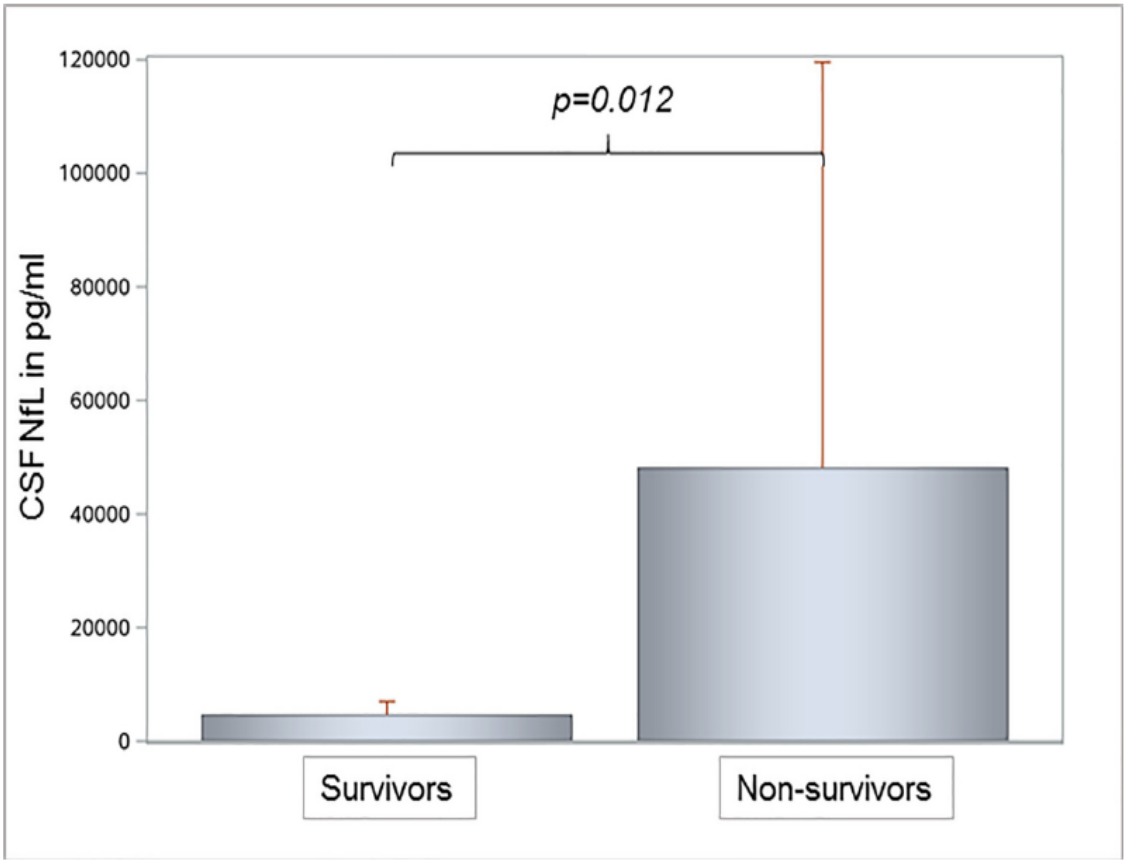

Fig 5. Cerebrospinal fluid neurofilament light chain levels in seven survivors and five non-survivors of sepsis. Significantly higher NFL levels were observed in non-survivors compared to survivors of sepsis. NfL Neurofilament light chain.

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Table 6. Spearmen's correlation analysis for cerebrospinal fluid and plasma NfH and NfL values.

Parameter	Cerebrospinal fluid		Plasma	
	NfH	NfL	NfH	NfL
BI before sepsis	R = -0.490 **p = 0.0028**	p>0.05	p>0.05	p>0.05
BI at day 100	p>0.05	R = -0.749 **p<0.0001**	p>0.05	R = -0.535 **p = 0.0003**
Days on ICU	p>0.05	p>0.05	p>0.05	p>0.05
Days in hospital	R = 0.571 **p = 0.007**	p>0.05	p>0.05	p>0.05
Days on ventilator	p>0.05	p>0.05	p>0.05	p>0.05
Days until death of non-survivors	R = -0.657 **p = 0.011**	R = -0.932 **p<0.0001**	R = -0.658 **p = 0.011**	p>0.05

BI Barthel index, *ICU* Intensive care unit, *NfH* Neurofilament heavy chains, *NfL* Neurofilament light chains, *n.s.* not significant, *SOFA* Sepsis-related organ failure assessment.

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to be controversial [19,48,49]. Our group is first to analyze the prognostic value of CSF and plasma NfH and NfL levels in SAE patients which might have importance for the prediction of long-term neurological sequelae and survival in sepsis. Biomarker research on SAE is necessary as most septic shock patients are sedated and mechanically ventilated and are not easily assessed for clinical signs of SAE [6,16,43]. Diagnostic measures to evaluate the extent of septic brain injury, as cerebral MRI or diffusion tensor imaging (DTI), are not universally available and require resources [50,51]. Specific markers of neuroaxonal injury in SAE could help to monitor SAE and to predict neurological outcome.

The highly elevated Nf levels in patients with SAE impressively underline the occurrence of brain injury in sepsis patients [9,10,13,52,53]. As we performed lumbar puncture early during the course of sepsis a close temporal relationship between the occurrence of septic shock and septic brain injury has to be suspected. The extent of brain injury was confirmed by MRI during study follow-up, which was significantly correlated to elevated NfL levels. Both, MRI and Nf results support an early start of sepsis treatment and a rapid hemodynamic stabilization of septic shock patients to prevent CNS and multiple organ failure. This is strikingly obvious with a look at the correlation of CSF Nf levels with time to death in non-survivors. Additionally CSF and plasma NfL levels significantly correlated with BI at day 100. Higher NfL values were associated with a poorer long-term functional outcome of survivors, which underlines the relevance of SAE and the prognostic value of NfL levels. We did not find a correlation between BI before sepsis and BI at day 100 after sepsis which might have been seen otherwise as a confounding factor.

Some limitations of the present study have to be mentioned. The number of study participants was low, which was a consequence of our strict exclusion criteria (a combination of peripheral and central nervous system diseases) for this single-center study. As SAE is a diagnosis of exclusion, we did not include patients with a preexisting neurological disease to prevent a main inclusion bias and to be able to evaluate the development of Nf levels in SAE over time as precise as possible. All patients finally included were otherwise examined by multimodal diagnostics. Our standard MRI examinations might still have underestimated the extent of brain injury of SAE patients. DTI, which was not available for our investigation would have been more accurate to visualize neuroaxonal injury [50].

The temporal relationship between onset and progression of sepsis and development of brain injury is supported by the longitudinal profile of the plasma Nf levels presented here. No

differences of Nf levels were measured at study day 1 between sepsis and control patients, which is in agreement with earlier studies showing that NfL is a slow marker reaching its maximum 10–14 days following traumatic brain injury [54]. This is also important as the comparable basal Nf levels were not primarily different between both groups, which could have been a confounder. Over time, the NfL levels increased in SAE patients, particularly so in patients with a positive CAM-ICU and severe SAE as indicated by ICDSC. This is relevant as plasma Nf might act as biomarkers to detect and monitor SAE in septic shock patients. NfL levels in CSF and by tendency in plasma were significantly higher in patients with brain injury seen on MRI. Recently our group demonstrated evidence for two distinct patterns of neuroaxonal injury in sepsis with ischemia and diffuse axonal injury as relevant pathomechanisms [9]. The results of the present study support the diagnostic role of Nf measurements to detect brain injury in sepsis and might support their suitability as potential biomarkers of neuroaxonal injury in SAE patients. This is supported by previous immunohistochemistry findings from human septic brain tissue [9]. We reported on the disruption of white matter axons in post-mortem brains of sepsis patients indicated by staining for nonphosphorylated NfH chains [9]. Immunohistochemistry gave clear evidence for axonal degeneration in sepsis which supports the diagnostic role of Nf measurements in SAE patients.

Conclusion

This is the first study on the relevance of neurofilament heavy (NfH) and neurofilament light chains (NfL) in cerebrospinal fluid (CSF) and plasma to detect SAE and to predict outcome in patients with sepsis. This prospective, longitudinal, registered study showed that NfL and NfH levels were found to be highly elevated in plasma and CSF of patients with SAE. Nf levels in sepsis correlated with the clinical appearance of SAE, the extent of neuroaxonal injury seen on MRI and with survival. Power calculations indicate that future studies on prediction of sepsis survival will require larger sample sizes compared to studies focused on cognitive/functional outcome in survivors. Given the difficulty in obtaining CSF samples in septic shock patients, the modest gain for study size calculation and the methodological developments we suggest future studies to focus on longitudinal plasma NfL and NfH levels using fourth generation immunoassays.

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CORRECTION

Correction: The prognostic value of neurofilament levels in patients with sepsis-associated encephalopathy - A prospective, pilot observational study

Johannes Ehler, Axel Petzold, Matthias Wittstock, Stephan Kolbaske, Martin Gloger, Jörg Henschel, Amanda Heslegrave, Henrik Zetterberg, Michael P. Lunn, Paulus S. Rommer, Annette Grossmann, Tarek Sharshar, Georg Richter, Gabriele Nöldge-Schomburg, Martin Sauer

In Fig 3, the headings above the graph are incorrectly swapped. The left heading should be "No Brain Dysfunction" and the right heading should be "Brain Dysfunction." The authors have provided a corrected version here.

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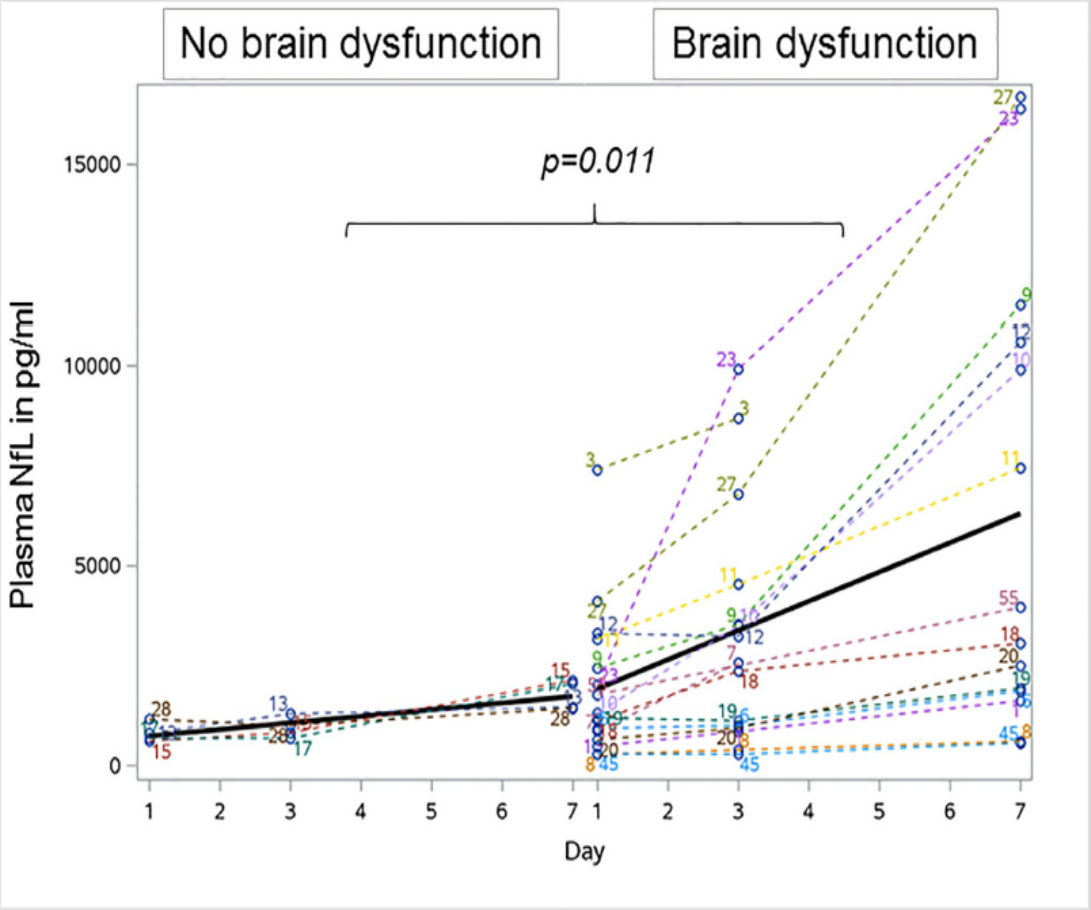

Fig 3. Longitudinal profile of plasma neurofilament light chain levels in 16 sepsis patients with brain dysfunction and four patients without brain dysfunction. NfL levels significantly increased in patients with brain dysfunction over time which was not observed in patients without brain dysfunction. Bold line indicates the development of mean plasma neurofilament levels over time. NfL Neurofilament light.

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1. Ehler J, Petzold A, Wittstock M, Kolbaske S, Gloger M, Henschel J, et al. (2019) The prognostic value of neurofilament levels in patients with sepsis-associated encephalopathy–A prospective, pilot observational study. PLoS ONE 14(1): e0211184. https://doi.org/10.1371/journal.pone.0211184 PMID: 30677080