Justus-Liebig-Universität Gießen

Fachbereich Medizin

Klinik und Poliklinik für Neurologie

Leiter: Prof. Dr. H.B. Huttner, PhD

Experimentelle und klinische Behandlungsansätze zur Verbesserung des Behandlungserfolgs beim ischämischen Schlaganfall

Habilitationsschrift

zur Erlangung der Lehrbefähigung für das Fach Neurologie

im Fachbereich Medizin der Justus-Liebig-Universität Gießen

vorgelegt von

Dr. med. Philipp Tobias Braun

Gießen (2021)

1. Inhaltsverzeichnis

2. Einführung

Nach der koronaren Herzkrankheit stellt der Schlaganfall die zweithäufigste Todesursache weltweit und die Hauptursache für bleibende Behinderungen bei Erwachsenen dar. Im Jahr 2004 starben weltweit 5,7 Millionen Menschen an einem Schlaganfall; dies entspricht9,7 Prozent aller Todesfälle[1,2]. Die Inzidenz in Deutschland wird auf 292/100.000 Einwohner geschätzt[3]. Der Schlaganfall kann durch eine intrazerebrale Blutung (ca. 15-20 Prozent der Fälle) oder durch ein ischämisches Ereignis, bedingt durch Thrombosen oder durch arterio-arterielle bzw. kardiale Embolien, ausgelöst werden[4].

2.1. Pathophysiologie des ischämischen Schlaganfalls

Das Gehirn ist auf eine kontinuierliche Versorgung mit Blut angewiesen, da es nicht über größere Energiespeicher verfügt. Zirka 20 Prozent des Herzminutenvolumens sind für die Aufrechterhaltung des Hirnstoffwechsels notwendig. Unter physiologischen Bedingungen wird das gesamte Gehirn ungefähr mit 60-80ml/100g/min Blut versorgt[5], dabei benötigt die graue Substanz etwa 80ml/100g/min und die weiße Substanz ungefähr 25ml/100g/min[6]. Sinkt die Durchblutung des Gehirns unter 20ml/100g/min (Ischämieschwelle) kann der Funktionsstoffwechsel, der die genuine neuronale Aktivität bedingt, nicht mehr aufrechterhalten werden. Das Membranpotential bricht zusammen, Kaliumionen strömen in den Extrazellularraum und Natrium- und Kalziumionen reichern sich intrazellulär an. Es kommt zur Depolarisation der Zellmembran, wodurch die Neurone nicht mehr erregbar sind. Dies bedingt die klinische Ausfallsymptomatik. Der Strukturstoffwechsel, der die Zellstruktur aufrechterhält, bleibt bis zu einer Schwelle von 10ml/100g/min intakt. Unterhalb dieser Schwelle (Infarktschwelle) kommt es bereits nach 4-5 Minuten zu einem irreversiblen Funktionsverlust der Neurone[5,7]. Relativ rasch bildet sich im Zentrum des betroffenen Areals, dem Infarktkern, eine Nekrose aus.

Um den Infarktkern herum ist die Durchblutung zwar vermindert, wird aber durch Kollaterale noch aufrechterhalten. Die Durchblutung liegt oberhalb der Infarkt- aber unterhalb der Ischämieschwelle. Damit besteht noch kein irreversibler Schaden[8,9]. Die Funktion der Nervenzellen in diesem Gebiet ist zwar gestört, jedoch ist der Strukturstoffwechsel zum Erhalt der Zellintegrität noch nicht zum Erliegen gekommen. Damit ist das Gewebe potentiell, durch Wiederherstellung des lokalen Blutflusses, zu retten[10,11]. Dieses Gebiet wird als „Penumbra“ (aus dem Lateinischen für „Halbschatten“) bezeichnet und ist das Ziel für rekanalisierende und neuroprotektive Therapiemaßnahmen in der akuten Phase des Schlaganfalls[12]. Wird der Blutfluss innerhalb einer gewissen Zeit nicht wieder hergestellt, so kommt es auch im Gebiet der Penumbra durch sekundäres Infarktwachstum zur Nekrose der Neurone[6,7].

Die Mechanismen des Zelluntergangs laufen sowohl kaskadenartig als auch parallel ab und verstärken sich zum Teil untereinander. Dieser Prozess wird als „postischämische Kaskade“ bezeichnet. Durch die dabei ablaufenden Mechanismen, zu denen Exzitotoxizität, Radikalbildung, Entzündungsreaktionen und Apoptose gehören, kommt es zur Schädigung des Penumbra-Gebietes und somit zur zentripetalen Ausweitung des ischämischen Areals[13,14].

Zudem kommt es im Rahmen der postischämischen Kaskade auch zu einer erhöhten Radikalbildung, u.a. durch die Aktivierung von Peroxidasen. Die hochreaktiven Sauerstoffspezies, wie H_2O_2 (Wasserstoffperoxid) und NO (Stickstoffmonoxid) führen durch oxidative Prozesse zur Schädigung von Lipiden, Proteinen und Nukleinsäuren, was wiederum zum Zelluntergang beiträgt[10]. Die hochreaktiven Sauerstoffspezies und die Freisetzung von Proteasen führen, durch Schädigung der extrazellulären Matrix, zur Störung der Integrität der Blut-Hirn-Schranke (BHS), was die Ausbildung des vasogenen Ödems zur Folge hat[14,15]. Die vasogene Ödemausbildung beginnt bereits in den ersten Stunden nach dem Infarkt und hat ihre größte Ausdehnung etwa am vierten Tag[16,17].

Das vasogene Ödem selbst führt wiederum zu raumfordernden Effekten, welchedie Durchblutung des umliegenden Gewebes vermindern und damit zu neuen Ischämien führen[18-21]. Bei ausgeprägtem vasogenen Ödem bei großen Hirninfarkten kann das Ödem massiv raumfordernd wirken (sog. „maligner Hirninfarkt“). Dies kann zur Einklemmung und damit zum Tod führen[22,23]. Durch Rettung der Penumbra sollen auch sekundäre Schäden wie die Ödembildung verhindert werden[24].

2.2. Akuttherapie des ischämischen Schlaganfalls

In der Akutphase des ischämischen Hirninfarkts ist das Ziel der Therapie die schnelle Wiedereröffnung des verschlossenen Gefäßes. Hierdurch soll die Durchblutung der Penumbra wiederhergestellt werden und das Ausmaß des irreversibel geschädigten Infarktkerns begrenzt werden[12,5]. Gemäß den aktuellen Leitlinien zur Akuttherapie des ischämischen Schlaganfalls kann eine Thrombolyse mit rekombinantem tissue-type Plasminogenaktivator (rt-PA) innerhalb der ersten 4,5 Stunden nach dem Symptombeginn erfolgen[25]. Die Sicherheit und Wirksamkeit dieser Therapie gilt als nachgewiesen[26,27]. Außerhalb dieses „Zeitfensters“ steigt die Häufigkeit von Blutungskomplikationen.

In den letzten Jahren konnte zusätzlich die Wirksamkeit der interventionellen Wiedereröffnung eines verschlossenen Gefäßes in Kombination mit rt-PA oder ohne rt-PA nachgewiesen werden. Seit der Veröffentlichung der Ergebnisse der wegweisenden Studien zur Thrombektomie (MR-CLEAN[28], ESCAPE[29], EXTEND-IA[30], SWIFT-PRIME[31], REVASCAT[32]) im Jahr 2016 und einer Metaanalyse dieser Studien[33] findet die Therapie in spezialisierten Zentren eine breite Anwendung. Diese Intervention steht auch außerhalb des 4,5-Stunden-

Zeitfensters zur Verfügung, wenn spezielle Bildgebung zur Darstellung von potenziell rettbarem Hirngewebe und damit zur Patientenselektion herangezogen wird. Dies kann mittels Perfusions-Bildgebung in der Computertomographie (CT) oder in der Magnetresonanztomographie (MRT) erfolgen. Hierbei stellt ein Unterschied („mismatch“) zwischen zerebralem regionalem Blutfluss (CBF) und zerebralem regionalem Blutvolumen (CBV) potenziell rettbares Gewebe dar (CBV/CBF-mismatch). Ebenso kann die strukturelle Hirnbildgebung mit dem MRT noch nicht irreversibel geschädigtes Gewebe nachweisen, da sich in diffusionsgewichteten Sequenzen eine Ischämie kurz nach Symptombeginn zeigt, in FLAIR-gewichteten Sequenzen (fluid attenuated inversion recovery) jedoch erst nach 4-6 Stunden (DWI-FLAIR-mismatch). Auch ist von rettbarem Gewebe bei weitgehend unauffälliger CT-Bildgebung und hoher Krankheitsschwere auszugehen (klinisch-bildgebendes mismatch). Unter Berücksichtigung des Blutungsrisikos bei größeren bereits demarkierten Infarkten kann auch gegebenenfalls eine systemische intravenöse Thrombolyse ergänzt werden. In allen mismatch-Situationen konnte zuletzt eine Überlegenheit der Thrombolyse und Thrombektomienachgewiesen werden[34].

2.3. Therapie des Schlaganfalls in der Post-Akut-Phase

2.3.1. Stroke Unit-Therapie

Den Grundpfeiler der Schlaganfalltherapie stellt das Stroke Unit-Konzept dar. Auf einer spezialisierten Schlaganfallstation, der Stroke Unit, werden die Diagnostik, die Akuttherapie, die Sekundärprophylaxe und die frühe Rehabilitationsbehandlung koordiniert. Abhängig von der Krankenhausstruktur kann die Akutdiagnostik und -therapie auch in einer Notaufnahmeeinrichtung erfolgen. Grundlage des Stroke Unit-Konzepts ist eine strukturierte, interdisziplinäre Zusammenarbeit von Neurologen, Internisten, Neurochirurgen, Logopäden, Ergo- und Physiotherapeuten, Pflegepersonal und dem Rettungsdienst. Allein die Behandlung auf einer solchen Stroke Unit senkt die Mortalität relativ um 18-46 Prozent (absolut 3 Prozent), das Risiko für eine funktionelle Abhängigkeit um 29 Prozent und die Notwendigkeit einer Betreuung im Pflegeheim oder eine vollständige häusliche Pflege um 25 Prozent[35]. Wesentlicher Wirkmechanismus scheint u.a. die sorgfältige Einstellung der Vitalparameter zu sein. Abweichungen von Körpertemperatur, Blutzucker und Blutdruck führen zu einem schlechteren klinischen Ergebnis[36-39]. Außerdem sollten Patienten frühzeitig auf Dysphagien, also Schluckstörungen, gescreent und die Ernährung angepasst werden[36].

2.3.2. Dysphagie – Eine häufige und schwerwiegende Komplikation beim Schlaganfall

Eine Dysphagie wird bei bis zu 80% der Schlaganfallpatienten der Akutphase als unmittelbare Folge des Hirninfarktes beschrieben[40,41]. Dabei unterscheidet man eine akute schlaganfallbedingte Dysphagie, die sich innerhalb von Tagen bis zwei Wochen zurückbildet, von einer chronischen schlaganfallbedingten Dysphagie, die darüber hinaus anhält und bei ca. 15-25% der Patienten auftritt[42]. Dysphagien führen häufig zu Aspirationspneumonien, die auf Stroke Units die wichtigste Sekundärkomplikation und die häufigste Todesursache beim Schlaganfall darstellen[40,43-45]. Innerhalb eines Jahres nach Schlaganfall sterben etwa 20% der Dysphagiepatienten an den Folgen einer Aspirationspneumonie[46]. Weitere dysphagieassoziierte Faktoren, die das Outcome negativ beeinflussen, sind die Hyperthermie (durch ein pneumoniebedingtes Fieber) sowie eine neue bzw. vorbestehende Mangelernährung, die durch die Dysphagie bedingt oder verstärkt werden kann[47,48]. Insgesamt ist die Pneumonie ein unabhängiger Prädiktor für Behinderung und ein schlechtes funktionelles Ergebnis, erhöhte Mortalität und eine deutliche Einschränkung der Lebensqualität[49-52]. Die langfristigen Folgen mit Pflegebedürftigkeit und hohen Folgekosten für das Gesundheitssystem unterstreichen die hohe sozioökonomische Relevanz der Dysphagie[48,53].

Aus den genannten Gründen ist das primäre Ziel der logopädischen Versorgung von Schlaganfallpatienten ist, neben Vermeidung von Malnutrition und Dehydrierung, insbesondere die Verhinderung des Auftretens einer Aspirationspneumonie. Die klinische und apparative Untersuchung des Schluckvorgangs innerhalb der ersten 24 Stunden nach dem Schlaganfall gehört daher zur Standarddiagnostik auf Stroke Units.

2.3.3. Zentrale Kontrolle des Schluckens

Die Dysphagieforschung der letzten Jahrzehnte konnte zeigen, dass das Schlucken nicht nur auf der Ebene des Hirnstamms abläuft. Das Schlucken wird von zahlreichen Regionen des Großhirns und des Hirnstamms, insbesondere der Medulla oblongata, gesteuert. Es existiert ein komplexes supramedulläres Netzwerk, welches je nach Situation (z.B. bewusstes vs. unbewusstes Schlucken) den Schluckvorgang modulieren kann. Die Hauptregion des Schluckens (nämlich den „Schluckkortex“ im engeren Sinne) stellt dabei die Region des inferioren Teils des prämotorischen, primär-motorischen und primär-sensorischen Kortex dar; dieser Bereich wird als kortikales Schluckzentrum im engeren Sinne bezeichnet und ist weitgehend identisch mit dem frontoparietalen Operculum. Eine weitere wichtige Region, die insbesondere beim willkürlichen Schlucken aktiv ist, ist die vordere Inselrinde. Außerdem sind unter anderem noch das supplementärmotorische Areal, die Basalganglien, der Thalamus, der Gyrus cinguli und das Kleinhirn beteiligt. Vom Schluckkortex projizieren Nervenfasern zur Medulla oblongata, sowohl gekreuzt als auch ungekreuzt. Von einer Kortexhälfte (der schluckdominanten Seite) zieht dabei eine größere Zahl an Nervenfasern zum Hirnstamm.

Man vermutet, dass sich die Seitendominanz unabhängig von der Händigkeit und der Sprachdominanz im Laufe des Lebens ausbildet[54-56].

Das weit verzweigte supramedulläre Netzwerk zur Kontrolle des Schluckens erklärt die hohe Prävalenz von Schluckstörungen beim Schlaganfall. Da prinzipiell jede Störung des Netzwerks zu einem Zusammenbruch desselben führen kann, sind die Schluckstörungen bei Schlaganfällen ein häufiges Symptom. Häufig beobachtet man jedoch spontane Erholungen der Schluckfunktion, da die ischämische Läsion nicht schluckrelevante Areale betrifft, sondern die Läsion eine „Fernwirkung“ auf das Netzwerk besitzt. Das Netzwerk kann sich in der Postakutphase erholen und die Funktion wieder aufnehmen[57]. Dieses Phänomen wird als Diaschisis bezeichnet und wird beispielsweise auch bei aphasischen Störungen beobachtet[58]. Über diesen Mechanismus lässt sich die prinzipiell gute Prognose der schlaganfallbedingten Dysphagie mit häufiger Spontanerholung erklären.

In der Medulla oblongata liegen die Hirnnervenkerne der am Schlucken beteiligten Hirnnerven IX, X und XII sowie die sogenannten Mustergeneratoren (Central Pattern Generators [CPGs]) des Schluckens. CPGs sind Teil der Formatio reticularis des Hirnstamms und stimmen die Funktionen relevanter Hirnnervenkerne untereinander ab; Beispiele sind Mustergeneratoren der Atmung, der Lokomotion, der Miktion und des Schluckens.

Tierexperimentell konnte man in jeder Hälfte der Medulla oblongata zwei CPGs identifizieren[59]: Ein dorsomedialer CPG (neben dem Nucleus tractus solitarii), der für die zentralnervöse Kontrolle des Schluckens verantwortlich ist (räumlich-zeitliche Koordination der Schluckmuskeln), und ein ventrolateraler CPG (neben dem Nucleus ambiguus), der den zeitlich-sequenzierten Output des dorsomedialen Mustergenerators verarbeitet und zu den schluckrelevanten Hirnnervenkernen weiterleitet. Die dorsomedialen CPGs erhalten dabei Input von sensiblen und sensorischen Hirnnerven und unterstehen zusätzlich der Kontrolle des supramedullären Schlucknetzwerkes[56]. Ischämien der dorsolateralen Medulla oblongata (Wallenberg-Syndrom) führen zum Teil zu schwersten Schluckstörungen, da hier immer der ventrolaterale CPG und oft auch der dorsomediale CPGs einer Seite betroffen sind[55].

2.3.4. Physiologischer und gestörter Ablauf des Schluckens

Der Schluckreflex selbst gilt als einer der komplexesten Reflexe des Menschen. An seinem Ablauf sind 5 Hirnnerven und 48 Muskelpaare direkt beteiligt. Üblicherweise wird der Schluckvorgang in 4 Phasen unterteilt: Die orale Vorbereitungsphase, die orale Transportphase, die pharyngeale Phase und die ösophageale Phase.

In der *oralen Phase* wird der Bolus zerkleinert, geformt und auf dem Zungenrücken (der sog. Zungenschüssel) gesammelt. Die orale Phase ist von individueller Dauer und willkürlich

steuerbar. Die *orale Transportphase* kann willkürlich initiiert werden. Dabei wird mittels peristaltischer Wellen („Zungenperistaltik“) der Bolus in Richtung Pharynx transportiert. Kontaktiert der Bolus den Bereich der Gaumenbögen, beginnt die *pharyngeale Phase*. Hierbei wird der Bolus unter Schutz der Atemwege in den Ösophagus transportiert. Während dieser Phase läuft eine auf die Boluseigenschaften abgestimmte Bewegungssequenz ab. Das Hyoid wird durch die suprahyoidale Muskulatur nach anterior und kranial bewegt und der Zungengrund bewegt sich in Richtung Rachenhinterwand. Die Kombination dieser beiden Bewegungen bedingt eine Inversion der Epiglottis, die den Eingang des Larynx verschließt. Zusätzlich kommt es zu einem Schluss der Taschenfalten und Stimmlippen. Das Gaumensegel wird angehoben und verschließt so den Mesopharynx in Richtung des Epipharynx. Da durch den Verschluss des Larynx und des Epipharynx ein Fluss der Atemluft nicht mehr möglich ist, kommt es zu einem funktionellen Stopp der Atmung (Schluck-Apnoe), was ein eventuelles „Ansaugen“ von Bolusbestandteilen in den Larynx verhindert. Die Hyoidhebung bedingt zusätzlich ein passives Aufdehnen und somit eine Öffnung des oberen Ösophagussphinkters, da das Skelett des Larynx mit dem Hyoid verbunden ist. Der obere Ösophagussphinkter selbst weist einen Dauertonus auf und kann lediglich „aktiv“ relaxieren. Das Aufdehnen erfolgt wie beschrieben passiv. Durch die Öffnung des Sphinkters kann der Nahrungsbolus in den Ösophagus eintreten und mittels Peristaltik durch diesen hindurch transportiert werden. Dabei gleitet der Bolus über die invertierte Epiglottis. In der *ösophagealen Phase* wird der Bolus mittels Peristaltik weiter in den Magen transportiert[56].

In allen Phasen des Schluckvorgangs sind Störungen möglich. Insbesondere die pharyngeale Phase ist vulnerabel für Störungen: Zentrale Störungen können dazu führen, dass die notwendigen schützenden Reflexe bei Paresen der am Schluckmuskulatur nicht suffizient ausgelöst werden. Auch kann eine verminderte Sensibilität zu Verzögerungen des Schluckreflexes bis hin zum Fehlen der Schluckreflexauslösung führen. Bei Hirnstamminfarkten im Bereich der Medulla oblongata (z.B. im Rahmen des Wallenberg-Syndroms) wird häufig eine fehlende Öffnung des oberen Ösophagussphinkters beobachtet, sodass der Bolustreintritt in den oberen Ösophagussphinkter nicht mehr bzw. nicht mehr vollständig möglich ist (primäre Öffnungsstörung des oberen Ösophagussphinkters). Da der obere Ösophagussphinkter sich nicht aktiv öffnen kann, ist eine adäquate Hyoidbewegung notwendig. Ist diese nicht ausreichend, resultiert daraus eine sekundäre Öffnungsstörung.

Ein weiterer typischer Pathomechanismus der schlaganfallbedingten Dysphagie ist eine verminderte Boluskontrolle in der oralen Phase. Sie bedingt ein frühzeitiges Abgleiten des Bolus in den Meso- und Hypopharynx, was als Leaking bezeichnet wird.

Alle diese Pathomechanismen oder ihre Kombination können letztendlich zu einem Eindringen von Bolusanteilen in den Larynxeingang (Penetration) oder bis unterhalb der Stimmlippen

(Aspiration) führen. Sind die laryngeale Sensibilität oder der reflektorische und der willkürliche Hustenstoß vermindert, wird entweder nicht oder nicht ausreichend auf diesen Reiz reagiert und der Larynx oder die Trachea werden nicht ausreichend durch Räuspern oder Husten gereinigt. Findet keinerlei Reaktion auf eine Penetration oder Aspiration statt, spricht man von einer „stillen" Penetration oder „stillen Aspiration"[56]. Der typische Pathomechanismus der schlaganfallbedingten Dysphagie ist eine verminderte Boluskontrolle mit einem posterioren Leaking und verzögerter Schluckreflextriggerung. Hierdurch kommt es zu einem „Überlaufen" von Bolusanteilen aus den Spalträumen des Pharynx in den Larynx, oder zu einem direkten Eindringen von Bolusanteilen in den Larynx mit verspätetem Verschluss der Atemwege. Häufig werden dabei stille Penetrationen oder Aspirationen beobachtet. Diese Mechanismen sind nicht nur auf Nahrungs- und Flüssigkeitsbestandteile beschränkt, sondern gelten genauso für den Speichel des Patienten[56,60]. Wie oben erwähnt, erhöht die Aspiration von Nahrung, Flüssigkeiten oder Speichel das Risiko für eine Pneumonie deutlich (bis zu 11,5-fach)[41].

2.3.5. Diagnostik der Dysphagie

Schluckstörungen können durch verkürzte klinische Screening-Untersuchungen, durch eine ausführliche logopädische Untersuchung oder durch instrumentelle Methoden erfasst werden[56,60]. Da definitionsgemäß eine stille Aspiration oder Penetration ohne von außen sichtbare Zeichen erfolgt, kann eine solche Störung lediglich durch instrumentelle Diagnostik eine solche Störung erfasst werden. Der Goldstandard zur Diagnostik einer Dysphagie ist, neben der Videofluoroskopie des Schluckens (VFSS; eine Röntgenuntersuchung ähnlich dem Ösophagus-Breischluck), die flexible transnasale videoendoskopische Untersuchung des Schluckvorgangs (FEES= **F**lexible **e**ndoskopische **E**valuation des **S**chluckens; früher auch **F**iber**e**ndoskopische **E**valuation des **S**chluckens). Während des natürlichen Schluckvorgangs beurteilt der Untersucher bei der FEES direkt am Patientenbett anatomische Veränderungen, Funktionsstörungen der sichtbaren Strukturen (z.B. Recurrensparese), Speichelmanagement, Penetration und Aspiration von Nahrungsboli, um Rückschlüsse auf den zugrunde liegenden Pathomechanismus einer eventuellen Dysphagie gezogen werden. Die erhobenen Befunde ermöglichen die Planung einer individuellen logopädischen Dysphagietherapie sowie die Bestimmung der optimalen und sicheren Ernährungsweise[56]. 1988 wurde die FEES erstmals von Susan Langmore beschrieben[61]. 2001 wurde von ihr ein Standard-FEES-Untersuchungsprotokoll (Langmore-Standard) publiziert, das bis heute den Goldstandard zur Durchführung der FEES darstellt[62]. Die Untersuchung soll dabei die folgenden Punkte umfassen:

- Ruhebeobachtung (z.B. anatomische Veränderungen [Tumor, Asymmetrien])
- Funktionsprüfung (Überprüfung von Motorik und Sensibilität)
- Eigentliche Schluckuntersuchung mit Nahrung und Flüssigkeit

- Überprüfung der Effektivität therapeutischer Maßnahmen.

Die FEES-Untersuchung ist mittlerweile ein etablierter Standard, der zunehmend in neurologischen Kliniken Einzug findet[63].

Die VFSS als auch die FEES werden als komplementäre Verfahren angesehen. Die VFSS bietet dabei der Vorteil, alle Phasen des Schluckens beurteilen zu können. Jedoch kann die VFSS das Speichelmanagement des Patienten nicht beurteilen. Auch müssen die Patienten hierfür in eine radiologische Abteilung gebracht werden und müssen aufrecht sitzen können[56,60]. Die FEES kann direkt am Patientenbett und damit auch bei bettlägerigen Patienten durchgeführt werden. Auch ist keine Röntgenstrahlung notwendig. Ein Nachteil der FEES ist, dass lediglich die pharyngeale Phase beurteilt werden kann und das im Moment des Schluckens, das endoskopische Bild verlegt wird, da das Endoskop an die Rachenhinterwand gepresst wird. Üblicherweise wird die FEES von einem Logopäden und einem Arzt gemeinsam durchgeführt.

2.3.6. Therapie der Dysphagie

Die Therapie der Dysphagie umfasst restituierende, kompensatorische und adaptative Verfahren wie z.B. Kräftigung schluckrelevanter Muskulatur, Haltungsänderungen oder Kostanpassungen[56,64,65]. Ergänzend können auch medikamentöse Ansätze wie beispielsweise die Therapie mit Amantadin[66], Capsaicin[67] oder ACE-Hemmern[68] verfolgt werden. Für ACE-Hemmer liegen lediglich Wirksamkeitsnachweise bei asiatischen Patienten vor[66]. Bei bestimmten Störungsbildern nach Hirnstamminfarkten kann auch eine Botolinumtoxin-Injektion in den oberen Ösophagussphinkter sinnvoll sein[69]. Außerdem existieren experimentelle Ansätze wie die transkutane muskuläre Stimulation[70], die transkranielle repetitive Magnetstimulation[71] oder die transkranielle Gleichstromstimulation[72].

Eine weitere, seit wenigen Jahren zugelassene und kommerziell verfügbare, Therapie ist die pharyngeale Elektrostimulation (PES). Hierbei wird über eine transnasal eingebrachte Stimulationssonde im Pharynx mit definierten Parametern elektrisch die Schleimhaut des Pharynx stimuliert, was über die Anregung der kortikalen Plastizität zu einer Verbesserung der Schluckfunktion führen soll[73,74]. Weitere Wirkmechanismen sollen eine Fazilitation der Aktivierung kortikobulbärer Bahnen und eine Erhöhung des Neurotransmitters Substanz P im Speichel sein. Die Substanz P ist ein Neurotransmitter, der für die Kontrolle des Schluckens eine Rolle zu spielen scheint[75,76].

Nachdem die PES vielversprechende Ergebnisse in kleinen Studien gezeigt hatte, wurde die multizentrische STEPS-Studie durchgeführt, die zwar die Sicherheit der Durchführung der Therapie nachweisen konnte, jedoch zeigte sich kein Nachweis einer Outcome-relevanten Wirksamkeit. Als Grund für dieses Ergebnis wurden die niedrigen Stimulationsstärken

angeführt. Zusätzlich wurden auch Patienten mit leichtgradigen Störungen eingeschlossen, die üblicherweise noch als normal bzw. nicht-pathologisch gewertet werden[77].

2.4. Ziele der vorliegenden Arbeit

2.4.1. Alternative und komplementäre Ansätze für die Akuttherapie des Schlaganfalls

In Deutschland wurde die systemische intravenöse Thrombolyse 2020 mit rt-PA lediglich bei 16,4% der Patienten durchgeführt. Trotz der Pandemiebedingungen in diesem Jahr blieb im Vergleich zum Vorjahr die Zahl der behandelten Patienten ungefähr gleich[78]. Die Gründe für die niedrige Zahl der mit rt-PA behandelten Patienten sind, dass die Patienten häufig zu spät (außerhalb des „Zeitfensters“) ärztliche Hilfe aufsuchen, mit den Schlaganfallsymptomen am Morgen aufwachen (Wake-Up-Stroke) oder in der erstversorgenden Klinik die Thrombektomie und die notwendigen speziellen bildgebenden Verfahren nicht routinemäßig und flächendeckend verfügbar sind. Auch kann die Thrombektomie nur bei einem Verschluss proximaler Gefäße erfolgen, da ein Gefäß einen ausreichenden Durchmesser für den Interventionskatheter aufweisen muss.

Selbst wenn ein Patient für eine systemische Thrombolyse in Frage käme, existieren Kontraindikationen wie eine orale Antikoagulation, aktive Tumorerkrankungen, Gerinnungsstörungen oder eine kürzlich durchgeführte Operation. Von einer systemischen Thrombolyse profitiert außerdem nur ein Teil der Patienten. Aus den Studien lässt sich berechnen, dass etwa jeder siebte behandelte Patient zusätzlich den Schlaganfall frei von alltagsrelevanten Behinderungen übersteht, wenn er nach den Zulassungskriterien behandelt wird[79]. Diese Beschränkung der Effektivität hängt vor allem mit einer inkompletten Rekanalisationsrate und mit der Zeitdauer bis zur erfolgreichen Rekanalisation zusammen[80]. Eine vollständige Rekanalisation des betroffenen Gefäßes wird bei einer rt-PA-Lyse nur bei der Hälfte der behandelten Patienten beobachtet[81]. Sind große bzw. proximale Gefäße betroffen, führt rt-PA nur in 10% der Fälle zu einer erfolgreichen Rekanalisation[82-84]. Daher scheint die alleinige Therapie mit rt-PA an ihre Grenzen zu stoßen.

Die Thrombektomie kam 2019 bei 7,7% der Schlaganfallpatienten zum Einsatz[78] und ist damit auch nur für einen kleinen Teil der Patienten eine Behandlungsmöglichkeit. Die schnelle Rekanalisation stellt jedoch den wichtigsten Faktor für ein gutes klinisches Ergebnis dar[85].

Die Kontraindikationen sowie die begrenzte Wirksamkeit der intravenösen Thrombolyse und die begrenzte Verfügbarkeit der Thrombektomie rechtfertigen die Entwicklung neuer Therapieansätze für die Akutphase des ischämischen Schlaganfalls. Bevor eine neue Therapie in einem klinischen Versuch an Menschen getestet wird, müssen die Wirksamkeit und die Sicherheit der Therapie im Tierexperiment evaluiert werden.

2.4.2. Planimetrie

Bevor experimentelle Studien bewertet werden können, sollte zunächst die Reliabilität der Erfassung des primären Endpunktes untersucht werden, um mögliche untersucherbedingte oder systematische Fehler zu erkennen. Dadurch kann verhindert werden, dass eine Verzerrung der Ergebnisse entsteht bzw. übersehen wird. Üblicherweise wird in der experimentellen Schlaganfallforschung das Volumen des ischämischen Areals als Zielparameter bestimmt, da dieser Parameter im Vergleich zu neurologischen Defiziten beim Tier klarer zu erfassen und weniger anfällig für Störfaktoren ist[86].

Für den Kliniker ist die grobe Abschätzung des Hirninfarktvolumens wichtig, da hierdurch die Prognose, das Risiko für die Entwicklung eines malignen Infarktes oder das Einblutungsrisiko abgeschätzt werden können. Hierzu reichen in der Regel einfache Quantifizierungsmethoden aus[22,87,88]. In der tierexperimentellen Schlaganfallforschung müssen jedoch weit höhere Anforderungen an die Quantifikation des Infarktvolumens gestellt werden, da dieser Parameter einer der wichtigsten Endpunkte präklinischer Studien darstellt. Die näherungsweise Bestimmung des Volumens eines Körpers, wie zum Beispiel des Gehirns oder des Hirninfarktes selbst, kann durch verschiedene Methoden erfolgen.

Die Planimetrie mittels computergestützter Verfahren ist die am häufigsten zur Volumetrie genutzte Methode in der experimentellen Schlaganfallforschung[89]. Obwohl diese Methode breite Anwendung findet, existieren nur wenige Daten über die Reliabilität und Validität der Methode. Insbesondere wurde auch nicht weiter untersucht, wieviel Übung in der Methode ein Untersucher, der die Methode neu erlernt, benötigt. Daher untersuchten wir zu mehreren Zeitpunkten die Reliabilität der Planimetrie anhand des Lernerfolgs zweier Untersucher, die die Methode neu erlernten.

2.4.3. Therapeutischer Ultraschall zur Rekanalisation eines Gefäßverschlusses

Therapeutischer Ultraschall stellt eine interessante und aussichtsreiche Möglichkeit dar, die bestehenden Therapieansätze zur Rekanalisation zu erweitern. Wichtigster Ansatz ist eine Wirksamkeitssteigerung einer Thrombolyse mit rt-PA durch parallele Beschallung des verschlossenen Gefäßes mit transkraniell appliziertem Ultraschall (nicht-invasive,

transkranielle Sonothrombolyse [STL]). Als möglicher Wirkmechanismus wird ein ultraschallbedingter vermehrter Transport von rt-PA in den Thrombus angesehen[90].

Da die Technik rasch und einfach anzuwenden ist, bietet sich der Vorteil, dass potenziell sehr viele Patienten behandelt werden können. Die prinzipielle Wirksamkeit konnte in verschiedenen experimentellen und klinischen Studien belegt werden[91-94]. In den letzten Jahren rückte die nicht-invasive Ultraschall-Therapie zunehmend in den Fokus der Schlaganfallforschung.

Klinische Studien unter Verwendung zugelassener Diagnostikgeräte zeigten einen Nutzen der Ultraschall-Therapie gegenüber einer Monotherapie mit rt-PA[93,94]. Allerdings ist trotz dieser innovativen Therapieverfahren in vielen Fällen eine Rekanalisation nicht oder erst zeitverzögert herbeizuführen. So konnte in einer größeren klinischen Studie zum Einsatz der Sonothrombolyse die Rekanalisationsrate im Vergleich zu einer rt-PA-Monotherapie zwar signifikant gesteigert werden, lag jedoch zwei Stunden nach Therapiebeginn trotzdem nur bei 38% (bezogen auf komplette Rekanalisation des Gefäßes)[93].

Eine weitere Steigerung des Therapieeffektes lässt sich durch zusätzliche Gabe von gashaltigen Mikrobläschen („Echosignalverstärkern“) erreichen, die im Schallfeld zu Schwingungen angeregt werden oder zerplatzen, wodurch lokal weitere Energie freigesetzt wird[95,96]. Dieser Ansatz wird auch als „microbubble mediated sonothrombolysis“ (mmSTL) bezeichnet. Ein signifikanter therapeutischer Effekt wurde beispielsweise für das Mikrobläschenpräparat SonoVue (Fa. Bracco, Genf, Schweiz) nachgewiesen[96].

Ein interessantes Forschungsfeld innerhalb der Sonothrombolyse stellt die Weiterentwicklung der Echosignalverstärker hin zu speziellen Mikrobläschenpräparaten mit verbesserten therapeutischen Eigenschaften dar. Eine solche Entwicklung stellt das Mikrobläschenpräparat BR38 der Firma Bracco dar. Hierbei handelt es sich um eine Perfluorgas-haltige Substanz, die sich im Vergleich zu SonoVue durch eine erhöhte Stabilität und eine geringere Bläschengrößenverteilung auszeichnet. Experimentelle in vitro Arbeiten haben gezeigt, dass eine erhöhte Stabilität von Gasbläschen zu einer verbesserten Effizienz führen kann[95].

Bevor eine solche Substanz wie BR38 in einer klinischen Studie geprüft werden kann, ist jedoch ein tierexperimenteller Nachweis der Anwendungssicherheit und Effektivität im Schlaganfallmodell notwendig. Zu diesem Zweck wurden Sicherheit und Effektivität einer STL mit BR38 bei einem thromb-embolischen Schlaganfallmodell bei der Ratte in Kombination mit oder ohne rt-PA evaluiert. Die Infarktgröße wurde als Parameter der Effektivität und das Auftreten von Blutungskomplikationen zur Bewertung der Sicherheit gewählt.

2.4.4. Das Hirnödem als Ziel der Therapie in der Akutphase des Schlaganfalls

Ein anderer vielversprechender Ansatz zur Behandlung von Schlaganfällen liegt möglicherweise im Einsatz neuroprotektiver Pharmaka. Diese sollen dazu führen, dass das ischämische Gebiet begrenzt wird, damit es zu weniger neuronalen Schäden und letztlich zu einer geringeren langfristigen Behinderung des Patienten kommt. Zahlreiche Substanzen, wie zum Beispiel Kalziumantagonisten, Radikalfänger oder NMDA-Rezeptorantogonisten, wurden erfolgreich im Tiermodell getestet. Jedoch konnte keine Substanz bisher in klinischen Studien überzeugen[97,98], sodass weitere Forschung auf diesem Gebiet nicht zielführend erscheint.

Untersuchungen an Schlaganfallmodellen von Nagetieren[21,99-101] deuten darauf hin, dass die Entwicklung eines vasogenen Hirnödems innerhalb der hyperakuten Phase des Schlaganfalls (<6 h) einen signifikanten, möglicherweise unterschätzten, Einfluss auf das Fortschreiten der Ischämie haben kann, da die Schwellung des ischämischen Gewebes innerhalb der starren Schädelhöhle zu Beeinträchtigung der Mikrozirkulation im kritisch hypoperfundierten penumbralen Bereich führen kann. Folglich können Kollateralschäden durch den raumfordernden Effekt eines Infarktes im Stromgebiet der A. cerebri media bis zu 50 % der ischämischen Läsion ausmachen[21]. Therapeutische Maßnahmen, die auf eine Reduktion des zerebralen Ödems und der daraus resultierenden raumfordernden Wirkung in den frühen Stadien des Schlaganfalls wirken, können als indirekte oder "sekundäre" Neuroprotektiva wirken[21,102].

Eine Kraniektomie rettet nachweislich das Leben von Patienten mit großen raumfordernden territorialen Schlaganfällen. Hier droht durch das Hirnödem eine Herniation von Hirngewebe mit erhöhter Mortalität. In großen Studien konnte durch die Kraniektomie eine signifikante Reduktion der Sterblichkeit von 71 auf 22% erreicht werden[103,104]. Experimentelle Studien zum Effekt der Kraniektomie in einem Nagetiermodell bei experimentellem Verschluss der A. cerebri media (englisch medial cerebral artery occlusion; MCAO), berichten von einer signifikanten Reduktion der Infarktgröße, die hauptsächlich auf die Reduktion der mechanischen Kompression zurückgeführt wird[105,106].

Untersuchungen zu den Effekten von systemisch verabreichtem rekombinantem humanem Erythropoetin (rhEPO) vor einem transienter MCAO bei Nagetieren legen nahe, dass der neuroprotektive Effekt eher aus einer Minderung des Hirnödems resultiert als aus direkten antiapoptotischen Effekten auf Neurone[107]. Wir untersuchten daher die Hypothese, ob rhEPO, das vor transienter MCAO verabreicht wird, neuroprotektive Eigenschaften in der Frühphase des Schlaganfalls durch Reduktion des zerebralen Ödems besitzt. Damit primäre von sekundären neuroprotektiven Effekten unterschieden werden konnten, erfolgte eine bilaterale Kraniektomie vor der experimentellen MCAO. Hierdurch kann eine Erhöhung des intrakraniellen Drucks verhindert werden[21,102]. Wir erwarteten, dass eine deutliche

Ödemreduktion durch Erythropoietin zu ausgeprägten Gruppenunterschieden hinsichtlich Infarktgröße und Ödemvolumen in Abhängigkeit von der Intaktheit der Schädelkalotte führt.

2.4.5. Optimierung der Dysphagietherapie auf der Stroke Unit

Auch die Postakutphase der Behandlung auf der Stroke Unit bietet Ansatzpunkte für die Verbesserung der Therapie. Insbesondere die Dysphagie als häufigster Grund für eine erhöhte Mortalität und Morbidität bietet dabei ein großes Potential.

Obwohl die Diagnostik und Therapie der schlaganfallbedingten Dysphagie in den letzten Jahren zunehmend in den Fokus der Forschung gerückt sind, konnte eine Cochrane-Analyse aus dem Jahr 2018 keine Wirksamkeit einer Therapie auf das klinisch-funktionelle Outcome der Patienten nachweisen. Bemängelt wurden die schlechte bis allenfalls mäßige Qualität der durchgeführten Studien sowie die Heterogenität der gewählten Endpunkte und der durchgeführten Therapien[108]. Daher sind qualitativ hochwertige Studien mit definierten und standardisierten Outcome-Parametern notwendig, um letztendlich die Komplikationen mit zum Teil erheblichen Einschränkungen der Lebensqualität, erhöhter Mortalität und Morbidität für die Patienten zu reduzieren.

2.4.6. Standardisierte Erfassung der Ernährungsweise als Zielparameter in Dysphagiestudien

Schluckstörungen mit Einschränkungen der Ernährungsweise führen zu einem deutlichen Verlust an Lebensqualität. Da die Lebensqualität der Patienten zunehmend als Parameter des Behandlungserfolgs in den Mittelpunkt rückt, scheint eine Erfassung der funktionellen Schluckfähigkeit bei Entlassung nach der Akut- oder Rehabilitationsbehandlung sinnvoll. Die Beurteilung der Schluckfunktion ist bisher kein routinemäßig erfasster Parameter, beispielsweise im Rahmen von Qualitätssicherungsmaßnahmen. Sie wird bisher lediglich im Barthelindex (BI) mit einem binären Item („Überwachungspflichtige Schluckstörung“; ja/nein) erfasst. Dabei stellt das Ausmaß einer Schluckstörung jedoch ein breites Kontinuum dar.

Eine sinnvolle Beurteilungsskala sollte im klinischen Alltag oder in der Forschung anwendbar sein, die Schluckfunktion bzw. ihre Einschränkung sinnvoll bewerten und auch eine Beurteilung des Verlaufs und damit die Messung eines Therapieerfolgs ermöglichen.

Die Functional Oral Intake Scale (FOIS) wurde 2005 von Cary et al. entwickelt und ermöglicht die oben geforderten Punkte[109]. Die FOIS ist eine ordinalskalierte, siebenstufige Skala, die die funktionelle orale Nahrungs- und Flüssigkeitsaufnahme mit guter Validität und Reliabilität bewertet. Die Stufen 1-3 erfassen unterschiedliche Abstufungen nicht-oraler Ernährung und

die Stufen 4-7 eine vollständige orale Ernährung mit Flüssigkeiten und Nahrung in jeweils unterschiedlichen Abstufungen. Vorteile der FOIS sind unter anderem klar definierte Unterschiede zwischen den Skalenstufen und die hohe psychometrische Qualität[110]. Sie findet nicht nur Anwendung bei Schlaganfallpatienten, sondern wurde auch an Patienten mit Parkinson-Syndromen, Amyotropher Lateralsklerose, Kopf-Hals-Tumoren und bei pädiatrischen Patienten validiert[111-114].

Zwar existieren mit der Functional Outcome Swallowing Scale (FOSS)[115], der Food Intake Level Scale (FILS)[116], der Dysphagia Outcome and Severity Scale (DOSS)[117] ähnliche Skalen, diese besitzen jedoch eine geringere Reliabilität und Validität.

Sowohl in unterschiedlichen Studien als auch im klinischen Alltag zeigte sich die FOIS als ein gut geeignetes und praktikables Instrument zur Beurteilung der funktionellen Schluckfunktion. Aufgrund dessen wurde zunächst eine deutsche Übersetzung der FOIS angefertigt und anhand von FEES-Untersuchungen validiert. Alle FEES-Untersuchungen wurden nach dem weiter oben beschriebenen FEES-Protokoll durchgeführt. Die validierte deutsche Version der FOIS (FOIS-G) dient als Dysphagie-Outcome-Parameter für weitere Studien.

2.4.7. FEES – Möglichkeit zur objektiven Erfassung und Ernährungsanpassung

Einer der positiven Effekte der Stroke Unit-Therapie scheint die frühe Erkennung und die Verbesserung der Schluckfunktion zu sein, wodurch die Häufigkeit von Pneumonien, Mangelernährung und Dehydratation reduziert werden können. Da stille Penetrationen und Aspirationen bei Schlaganfallpatienten häufig vorkommen[118], ist apparative Diagnostik hier zwingend erforderlich.

In der klinischen Routine bietet die FEES zahlreiche Vorteile gegenüber der VFSS. Die FEES wird am Patientenbett und ohne eine Strahlenbelastung durchgeführt. Zusätzlich bietet die FEES den Vorteil einer genauen Evaluation des Speichelmanagements. Sie kann bei unkooperativen oder bewusstseinsgeminderten Patienten durchgeführt und beliebig oft wiederholt werden. Auch kann mittels FEES ein „natürlicher“ Schluckvorgang beurteilt werden. Für die VFSS muss der Patient für eine gewisse Zeit aufrecht sitzen können, ein Kontrastmittel ist notwendig und das Schlucken findet nur auf ein Kommando statt. Gegenüber der VFSS stellt die FEES in der klinischen Routine eine geringere Belastung für den Schlaganfallpatienten dar mit einer dennoch präzisen bildgebenden Evaluation der Dysphagie. Entsprechend der Fähigkeiten des Patienten wird anhande der FEES-Befund die Kostform angepasst und eine pathomechanismen-geleitete Therapie ausgewählt[56,60].

Da die Dysphagie für einen Schlaganfallpatienten eine deutliche Gefährdung sowie Einschränkung der Lebensqualität darstellt, untersuchten wir, ob Schlaganfallpatienten in

unserer Klinik die für sie adäquate Ernährung mit Nahrung und Flüssigkeit erhielten oder ob eine weitere Anpassung der Ernährung nach instrumenteller Diagnostik notwendig war. Dies würde darauf hinweisen, dass zwischen den Ergebnissen der klinischen und apparativen Diagnostik deutliche Unterschiede bestehen. Da bekannt ist, dass es bei einer Dysphagie zu erhöhter Mortalität und Morbidität sowie einer verlängerten Aufenthaltsdauer im Krankenhaus kommt, untersuchten wir zusätzlich, ob durch eine Anpassung der Ernährung die Aufenthaltsdauer in der Klinik sowie die Mortalität sinken. Auch wurde untersucht, ob das Behandlungsergebnis bei Patienten mit Anpassung der Ernährung zufriedenstellender ist. Insgesamt stellte sich also die Frage, ob die FEES bei Schlaganfallpatienten routinemäßig eingesetzt werden sollte und ob die daraus entstehenden Konsequenzen auch möglicherweise einen positiven Effekt für die Patienten haben.

2.4.8. Einsatzmöglichkeiten der FEES auf der neurologischen Intensivstation

Die gerade beschriebene Problematik trifft auch auf Patienten auf einer neurologischen Intensivstation zu. Ein Großteil der Patienten auf einer neurologischen Intensivstation wird aufgrund eines Schlaganfalls behandelt. Die Aspirationspneumonie ist bei intensivmedizinisch behandelten Patienten einer der stärksten Prädiktoren für ein schlechtes Patientenoutcome und eine erhöhte Mortalität.

Zusätzlich zu den schlaganfallspezifischen Störungen, die eine Dysphagie verursachen, kann hier auch eine Dysphagie bedingt durch die intensivmedizinische Therapie auftreten. Auslösende Faktoren können anatomische Veränderungen nach Intubation und Tracheotomie, eine Verminderung der Sensibilität der schluckrelevanten oropharyngealen Areale, eine verminderte Bewusstseinslage, ein gastroösophagealer Reflux oder eine Desynchronisation von Atmung und Schlucken sein[119]. Auch können muskuläre Schwächen aufgrund einer Critical-Illness-Polyneuropathie oder -Myopathie entstehen, die bei bis zu 75% der Patienten, die länger als 3 Wochen beatmet werden, auftreten[120].

Außerdem ist bekannt, dass eine vollständige orale Nahrungs- und Flüssigkeitskarenz (NPO; „nil per os“) bei Intensivpatienten zu einem schlechteren Behandlungsergebnis führt. Wir verglichen daher die Ergebnisse solcher Patienten mit Patienten, die zumindest geringe Mengen an Flüssigkeit und Nahrung oral erhielten[121,122]. Patienten ohne orale Ernährung sind prädisponiert für eine Mangelernährung, selbst wenn diese Patienten parenteral oder enteral mittels Magensonde ernährt werden[123,124].

Auch hier stellte sich die Frage, ob neurologische Intensivpatienten von einer FEES-Untersuchung profitieren und ob die Patienten adäquat ernährt werden. Es liegen bereits Studien zur FEES auf Intensivstationen vor. Der Fokus dieser Studien lag nicht auf

neurologischen Patienten, die FEES wurde nicht mit der klinischen Schluckuntersuchung verglichen und es wurden auch keine für das Behandlungsergebnis relevanten Parameter, wie die Pneumonierate oder die Mortalität berichtet[125,126].

Wir untersuchten daher auch hier, ob Unterschiede zwischen klinischer und apparativer Beurteilung bestehen und ob sich diese Patienten, die eine Anpassung der Ernährung erhielten, bezüglich Pneumonierate, Mortalität, Aufenthaltsdauer im Krankenhaus, Dauer der Maschinellen Beatmung und anderer Parameter unterschieden. Da Schlaganfallpatienten den Großteil der neurologischen Intensivpatienten stellen, erfolgte auch eine entsprechende Subgruppenanalyse. Da wie erwähnt NPO-Patienten ein schlechteres Behandlungsergebnis als Patienten mit zumindest geringen Nahrungs- oder Flüssigkeitsmengen haben, erfolgte zusätzlich auch entsprechende Subgruppenanalyse.

2.4.9. Pharyngeale Elektrostimulation zur Verbesserung der Schluckfunktion bei tracheotomierten Schlaganfallpatienten

Insbesondere intensivmedizinisch behandelte Schlaganfallpatienten haben ein erhöhtes Risiko an einer Dysphagie zu leiden. Ungefähr 25% der Schlaganfallpatienten, die auf einer Intensivstation behandelt werden, benötigen eine Tracheotomie aufgrund einer schweren Dysphagie[127-130]. Obwohl die Patienten in der akuten Phase des Schlaganfalls von der Tracheotomie profitieren[131], führt das Vorhandensein einer Trachealkanüle nach Entwöhnung von der Beatmung zu negativen Folgen bezüglich zeitgerechter Rehabilitationsbehandlung, Patientenkomfort, Aufenthaltsdauer im Krankenhaus[130], Wiederaufnahmen ins Krankenhaus[132] und erhöhten Behandlungskosten[132,133]. Auch ist das Vorhandensein einer Trachealkanüle prädiktiv für ein schlechteres Behandlungsergebnis, was zumindest in Teilen auf Komplikationen mit der Trachealkanüle selbst zurückzuführen ist[134,135]. Eine schwere Dysphagie stellt bei Schlaganfallpatienten den häufigsten Grund dar, warum diese Patienten drei Monate nach dem primären Ereignis nicht dekanüliert werden können[131,136].

Es existieren jedoch nur wenige Maßnahmen, die die Dauer der Entwöhnung von der Trachealkanüle verkürzen können[137]. Die PES konnte im Vergleich zu einer Scheinstimulation in einer Pilotstudie an 30 tracheotomierten Schlaganfallpatienten eine Verbesserung des Schluckens hervorrufen. Eine Dekanülierung konnte in 75% der Patienten mit PES erfolgen, während nur 20% der Kontrollpatienten dekanüliert werden konnten[138]. Aufgrund dieser vielversprechenden Ergebnisse nahmen wir an der multizentrischen PHAST-TRAC-Studie teil, in der diese Ergebnisse repliziert werden sollten.

2.5. Zusammenfassung der zu erarbeitenden Fragestellungen

Mit der routinemäßigen Anwendung der Thrombektomie steht uns nun eine äußerst wirksame Therapie in der Akutphase des Schlaganfalls zur Verfügung. Dennoch kommt diese Therapie nur bei einem kleinen Teil der Patienten zur Anwendung. Auch die systemische Thrombolyse wird zwar durch die Durchführung einer elaborierteren Bildgebung häufiger angewendet werden, jedoch existieren unverändert Kontraindikationen und auch ein begrenztes Zeitfenster, was ihren Einsatz weiterhin beschränkt. Auch die Kraniektomie, als weiteres evidenzbasiertes Verfahren, stellt nur in Ausnahmefällen eine Option dar und geht insbesondere bei Patienten mit einem Alter über 60 Jahren trotz Überlebens häufig mit einer deutlichen Behinderung einher[139].

In der Postakutphase stehen mit der Stroke Unit-Therapie (Number-needed-to-treat oder besser Number needed to care for 33[140]) und der Gabe von Acetylsalicylsäure (Number needed tot treat 79[141]) lediglich zwei Therapien mit gesicherter, jedoch nur mäßiger Wirksamkeit zur Verfügung.

Daher kommt der Suche nach einer Verbesserung der Behandlungsoptionen in der Akut- und der Postakutphase auf der Stroke Unit eine große Bedeutung zu. Die vorliegende Arbeit versucht in beiden Phasen zu einer Verbesserung beizutragen. In der Akutphase soll jeweils experimentell die Größe des Schlaganfalls begrenzt und damit das Behandlungsergebnis verbessert werden. Zum einen soll versucht werden, ob dies durch eine frühe Wiedereröffnung des verschlossenen Gefäßes mittels Sonothrombolyse oder zum anderen durch die Verminderung des postischämischen Hirnödems durch die Gabe von EPO erreicht werden kann. Bevor diese Studien bewertet werden können, soll jedoch zunächst die Reliabilität der Planimetrie, die zur Erfassung des primären Endpunktes der Studien dient, untersucht werden. Der klinische Teil der vorliegenden Arbeit versucht zu beantworten, ob eine instrumentelle Schluckdiagnostik einer klinischen Diagnostik überlegen ist und ob sich durch eine Anpassung der Ernährung nach Durchführung einer FEES-Untersuchung Komplikationen vermeiden lassen. Dies wurde nicht nur an Schlaganfallpatienten auf einer Stroke Unit, sondern auch bei kritisch kranken Patienten auf einer neurologischen Intensivstation inklusive einer Subgruppenanalyse für intensivpflichtige Schlaganfallpatienten überprüft. Da die Art der Ernährung mittels FOIS gemessen wurde, erfolgte zunächst die Validierung unserer deutschen Übersetzung der FOIS für die FEES. Zuletzt wurde untersucht, ob durch Verwendung der pharyngealen Elektrostimulation bei tracheotomierten Schlaganfallpatienten die Schluckfunktion gebessert und so eine frühere Dekanülierung nach abgeschlossener Entwöhnung von der Beatmung erfolgen kann.

3. Diskussion

3.1. Experimentelle Aspekte

3.1.1. Reliabilität der computergestützten Planimetrie beim experimentellen Hirninfarkt

Die computergestütze Planimetrie ist ein in der experimentellen Schlaganfallforschung häufig angewendetes Verfahren zur Bestimmung der Größe des Schlaganfalls als primärer Outcomeparameter. Der Einsatz computergestützter Verfahren in der experimentellen Schlaganfallforschung suggeriert ein gewisses Maß an Objektivität, dennoch muss bei der der Datenerfassung von einer gewissen Rater-Abhängigkeit ausgegangen werden. Daher untersuchten wir, ob es bei der computergestützten Planimetrie Rater-abhängige Variationen bezüglich der Messwerte gibt.

Wir konnten zeigen, dass das Inter-Rater-Agreement ein hohes Maß an Übereinstimmung nach etwa zwei Monaten oder 284 untersuchten MRT-Sequenzen erreichte und über einen Beobachtungszeitraum von 15 Monaten stabil blieb. Dies konnte durch Verwendung von Bland-Altman-Plots bestätigt werden. Auch zeigten sich keine Hinweise auf systematische Fehler

Es gibt nur wenige Daten über die Inter- und Intra-Rater-Reliabilität der Planimetrie in der experimentellen Schlaganfallforschung. Nagel et al. konnten eine gute Intra-Rater-Reliabilität bei der Anwendung der Planimetrie mit MRT-Sequenzen nachweisen, die abhängig vom Zeitraum zwischen experimenteller Infarktinduktion und der erfolgten Bildgebung war[142]. Friedländer et al. berichteten von einer geringeren Reliabilität der Planimetrie im Vergleich zu einem ImageJ-Makro bei Verwendung von Triphenyltetrazoliumchlorid-Färbung zur Darstellung des Infarktareals[143]. Allerdings waren die Untersucher in dieser Studie nicht in der Methode geschult.

Unsere Daten legen nahe, dass die computergestützte Planimetrie eine geeignete Methode zur Bestimmung des Hemisphären- oder ischämischen Läsionsvolumens bei Nagetieren sein kann, aber eine ausreichend lange Lernphase erforderlich ist. Doch auch nach dieser Lernphase bleibt die Methode anfällig für untersucherabhängige, systematische Fehler. Nach unserer Erfahrung ist dies vor allem auf unterschiedliche Kontrastparameter, Unschärfen an der Grenze zwischen ischämischer Läsion und gesundem Hirngewebe, sowie kleinen Abweichungen beim Umfahren der Grenzen des Objekts mit einer Computermaus, die zu Abweichungen infolge einer Fehlerfortpflanzung führen, zurückzuführen.

Die Validität der Planimetrie kann nicht sicher bestimmt werden, da es keinen Goldstandard gibt, mit dem das exakte Volumen der Hemisphäre bzw. der ischämischen Regionen quantifiziert werden kann.

Das Hemisphärenvolumen kann theoretisch durch Wasserverdrängung nach dem Archimedischen Prinzip bestimmt werden. Allerdings ist eine Übertragung dieser Methode auf die unregelmäßig und inhomogen geformte ischämische Läsion schwierig. Da eine Möglichkeit, die Validität dieser Methode zu testen, nur schwer zu realisieren ist, haben wir diesen Aspekt nicht weiterverfolgt.

Ein anderer, im klinischen Alltag etablierter Ansatz für die Volumetrie ist die Verwendung euklidischer Geometrie. Hierbei wird das Läsionsvolumen näherungsweise mit einfachen geometrischen Modellen berechnet (Kugel, Ellipsoid, Zylinder). Diese Modelle neigen zur Über- oder Unterschätzung des wahren Volumens der Läsion. Ein kugelförmiges Modell scheint die besten Ergebnisse im Vergleich zur Planimetrie zu erzielen[144]. Da jedoch ischämische Läsionen unregelmäßig geformt sind, stellen diese Modelle nur eine Vereinfachung dar.

Darüber hinaus gibt es verschiedene automatisierte Methoden zur Volumetrie: Das Läsionsvolumen kann mit Hilfe eines ADC-Schwellenwerts in MRT-Sequenzen[145] oder anhand von Grauwerten der MRT-Bilder mittels Autotracing[142] bestimmt werden. Beide Methoden sind schnell, automatisiert und benutzerunabhängig. Sie können jedoch nicht verwendet werden, wenn das Hemisphärenvolmen beurteilt werden soll. Daher sind ausreichende Fähigkeiten und Training der Planimetrie in der experimentellen Schlaganfallforschung erforderlich, wenn das Hemisphärenvolumen beurteilt werden soll.

Aufgrund unserer Beobachtung, dass die Intra-Rater-Übereinstimmung geringer war als die Inter-Rater-Übereinstimmung, scheint es plausibel, dass selbst ein erfahrener Untersucher Daten mit gravierenden Abweichungen erzeugen kann. Dies muss beim Studiendesign und der Interpretation planimetrischer Daten berücksichtigt werden. Eine alternative Interpretation wäre eine nur unzureichende Planimetrie-Fähigkeiten eines Untersuchers, obwohl er etwa 18 Monate Erfahrung in der Anwendung der Planimetrie hatte.

Zusammenfassend lässt sich sagen, dass die Planimetrie ein zuverlässiger Ansatz zur Volumetrie ist. Soll die Methode verwendet werden, sollten die Anwender ausreichend geschult werden. Eine ausreichende Einarbeitung in die Methode wurde bei den beiden folgenden Tierexperimentellen Studien sichergestellt.

3.1.2. Mikrobläschengestütze Sonothrombolyse als alternatives rekanalisierendes Verfahren

Die Sonothrombolyse stellt eine einfach anzuwendende Methode dar, die zu einer früheren Eröffnung eines Gefäßverschlusses führt. Die Wirkung kann durch die zusätzliche Gabe von rt-PA sowie die Anwendung eines Mikrobläschenpräparats verbessert werden. Wir untersuchten daher die Sicherheit und Wirksamkeit der STL mit Hilfe eines solchen Mikrobläschenpräparats (BR38 der Firma Bracco, Genf, Schweiz) in Kombination mit rt-PA, ohne rt-PA gegen rt-PA ohne Sonothrombolyse oder gegen eine reine Placebotherapie.

Unsere Studie zeigte bei allen Tieren ein geringeres Läsionsvolumen unter einer aktiven Therapie (STL, rt-PA oder STL+rt-PA) im Vergleich zur Placebobehandlung. Unterschiede zwischen den verschiedenen Behandlungsgruppen fanden wir dabei nicht.

Bezüglich der Untersuchung der Sicherheit der Sonothrombolyse mit Mikrobläschenpräparaten (mmSTL) zeigten sich bei allen Tieren in der Studie Mikroblutungen, d.h. auch in der Placebogruppe. Wir fanden keine Hinweise in der Häufigkeit von größeren intrazerebralen Blutungen, die mittels SWI-Sequenzen (Susceptibility-weighted imaging) im MRT nachgewiesen wurden. Unterschiede bezüglich der Mortalität zwischen den Gruppen lagen ebenfalls nicht vor. Tiere mit intrazerebraler Blutung unterschieden sich in den funktionellen Tests nicht von den Tieren ohne intrazerebrale Blutung. Da wir keine Unterschiede bei den intrazerebralen Blutungen zwischen den Gruppen sahen, scheint eine verzögerte Rekanalisation als Wirkmechanismus der STL unwahrscheinlich. Eine verzögerte Rekanalisation ginge mit einem Reperfusionsschaden und einer höheren Rate an intrazerebralen Blutungen einher[146].

Zum Zeitpunkt 90 Minuten nach MCAO sahen wir keine Unterschiede zwischen den Behandlungsgruppen. Dies ist vor allem darauf zurückzuführen, dass die T2-Bildgebung, die das zerebrale vasogene Ödem widerspiegelt, einige Zeit benötigt, um eine ischämische Läsion zu zeigen. Auch kann noch ungeschädigtes Hirngewebe vorhanden sein, das über Kollateralen versorgt wird. Diese können im Laufe der Zeit zusammenbrechen, was zu einem verzögerten Infarktwachstum führt. Das vasogene Ödem führt durch die kompressive Wirkung auf die Hirngefäße zu einem sekundären Infarktwachstum, entwickelt sich aber erst über die Zeit. Es ist für bis zu 50% des definitiven Infarktvolumens verantwortlich[21].

Hinsichtlich der Sicherheit der mmSTL konnte in keiner existierenden experimentellen in vivo Studie eine höhere Rate an Hirnblutungen festgestellt werden, unabhängig vom gewählten Schlaganfallmodell, dem Tier, der Verwendung von Mikroblasen oder der Verwendung eines begleitenden Thrombolytikums[147]. In den meisten Studien wurde die mmSTL als wirksam eingestuft. Allerdings wurden zahlreiche primäre Endpunkte wie Läsionsvolumen, Vorhandensein einer Rekanalisation, Zeit bis zur Auflösung des Thrombus, D-Dimer-Werte

oder klinische Scores verwendet. Weitere methodische Unterschiede betreffen das gewählte Tiermodell (Schwein, Ratte, Kaninchen), die Thrombuspräparation (autologer oder humaner Thrombus; rote oder weiße Thromben), den Weg der Mikrobläschenenverabreichung (arteriell oder venös), die gewählten Ultraschallparameter und die Verwendung von rt-PA[147]. Diese Heterogenität erschwert die Einordnung unserer Ergebnisse.

Nur 8 Studien untersuchten die mmSTL bei Ratten[96,148-154]. In 6 dieser Studien wurde die mmSTL mit rt-PA kombiniert[96,148-150,152,153]. Dabei zeigte sich insgesamt die Therapie mit rt-PA und mmSTL überlegen. Die Monotherapie mit mmSTL zeigte teilweise keine Rekanalisation.

Unsere Studie zeigte eine Wirksamkeit und Sicherheit der mmSTL mit oder ohne gleichzeitiger Verwendung von rt-PA. Es ist schwierig, unsere Ergebnisse mit denen einer ähnlichen Studie von Dixon et al. zu vergleichen. Sie berichteten über ein Volumen des Thrombus von 16 µl, das über einen PE-10-Katheter verabreicht wurde[153]. Der Durchmesser des PE-10-Katheters wurde nicht angegeben und variiert zwischen den verschiedenen Herstellern. Berechnet man die Länge des Thrombus bei einem angenommenen Innendurchmesser von 0,28 mm und geht man davon aus, dass das gesamte Volumen aus Thrombusmaterial besteht, würde dies einen etwa 26 cm langen Thrombus ergeben, welcher der Ratte verabreicht wurde.

Es ist wichtig zu beachten, dass wir nachweisen konnten, dass es einen therapeutischen Effekt von mmSTL ohne Verwendung von rt-PA gab. Lu et al. und Ren et al. zeigten eine Wirkung von mmSTL ohne rt-PA bei Ratten, verwendeten aber ein anderes Thrombusmodell[150,152]. Brown et al. konnten ebenfalls eine Wirkung von mmSTL allein zeigen, allerdings an Kaninchen[155]. In vitro-Studien haben nahegelegt, dass mäßige Ultraschallintensitäten nur einen begrenzten Effekt auf die Thrombolyse ohne Verwendung von rt-PA haben[156]. In der in vivo-Situation ist jedoch intrinsisches t-PA vorhanden, das einen thrombolytischen Effekt begünstigen könnte. Da rt-PA in seiner Anwendung durch Nebenwirkungen und Kontraindikationen beim Menschen beschränkt ist, gibt es also dennoch Hinweise auf eine ausreichende Wirksamkeit der mmSTL.

Zusammenfassend zeigte unsere Studie die Wirksamkeit und Sicherheit der mmSTL mit oder ohne gleichzeitiger Verwendung rt-PA in einem embolischen Schlaganfallmodell der Ratte mit einem kontinuierlichen Vollblutthrombus.

3.1.3. Erythropoietin-vermittelte Neuroprotektion

In der Vergangenheit wurde häufig versucht, durch die Beeinflussung von Stoffwechselwegen, ein geringeres Infarktvolumen nach einem Schlaganfall zu erreichen, was als Neuroprotektion bezeichnet wurde. Zahlreiche Experimente wurden diesbezüglich durchgeführt, konnten jedoch in klinischen Studien keine Wirksamkeit nachweisen. Eine weitere Möglichkeit das Infarktvolumen zu verringern, stellt die Therapie des vasogenen Hirnödems dar. Hierbei wird nicht direkt in die postischämische Kaskade eingegriffen. Vielmehr soll durch die Verminderung von Kompressionseffekten die Perfusion der Penumbra erhalten bleiben und so das Infarktwachstum begrenzt werden. Dieser Ansatz wurde von unserer Arbeitsgruppe als „indirekte“ oder „sekundäre Neuroprotektion“ bezeichnet. Für EPO wurde postuliert, dass EPO möglicherweise durch solche sekundären Effekte zu einem geringeren Infarktvolumen führt. Daher sollte untersucht werden, ob sich durch das Wegfallen von Kompressionseffekten bei bilateraler Kraniektomie weiterhin Effekte auf das Infarktvolumen bei einer Therapie mit EPO im Vergleich zu Placebo zeigten.

Wir beobachteten in unserer Studie, dass eine rhEPO-Behandlung vor dem transienten Verschluss der A. cerebri media die ödemkorrigierte Infarktgröße um etwa 10 % reduzierte. Daten zu Experimenten mit vergleichbarem Setting sind begrenzt; zwei frühere Studien an Ratten berichteten über keine signifikanten Effekte auf das Infarktvolumen bei EPO-Vorbehandlung. Im Gegensatz dazu zeigte eine Studie an Mäusen eine Infarktreduktion um bis zu 47%[107,157,158]. Interessanterweise konnte in unserer Studie eine signifikante Reduktion im Vergleich gegenüber der Placebobehandlung nur dann beobachtet werden, wenn der Schädel intakt gelassen wurde.

Die antiödematösen Effekte von EPO beim experimentellen Schlaganfall werden insbesondere auf eine erhaltene Barrierefunktion der Blut-Hirn-Schranke (BHS) zurückgeführt wird[159-164]. Eine Untersuchung zu Markern der BHS-Integrität - wie Occludin, alpha- und beta-Catenin - zeigte, dass eine EPO-Behandlung vor und 3 Tage nach fokaler zerebraler Ischämie die BHS stabilisieren, ihre Permeabilität reduzieren und dadurch zerebrale Entzündungen und Ödeme eingrenzen kann[162]. Die Permeabilität der BHS hängt hauptsächlich von intakten Endothelzellen und Tight Junctions ab, die in der Phase der Reperfusion nach transienter Ischämie durch die Bildung reaktiver Sauerstoffspezies und Lipidperoxidation einem erheblichen oxidativen Stress ausgesetzt sind[165,166]. Unter diesen Bedingungen scheint EPO die endotheliale Stickoxid-Produktion zu stimulieren und hat die Fähigkeit, die Reperfusions-vermittelte Schädigung der BHS zu vermindern[167]. Da das Läsionsvolumen proportional zum Wassergehalt der Hemisphären ist, kann das Infarktvolumen Methoden zur Quantifizierung des Wassergehalts des Gehirns, die die Hemisphären miteinschließen, verfälschen. Dies trifft beispielsweise auf die Nass-Trocken-Technik und die Bestimmung der Mittellinienverlagerung zu. Daher wurde das Hirnödem in der vorliegenden Untersuchung im MRT mittels Messung

der T2-Relaxationszeit (T2RT) in Regions of Interest (ROI) bestimmt, da sich diese Methode als weitgehend unabhängig von der Läsionsgröße erwiesen hat[100]. Die T2RT ist korreliert mit dem Ausmaß des vasogenen Ödems. Wir konnten eine signifikante Reduktion der Mittellinienverlagerung für die EPO-Vorbehandlung nur dann nachweisen, wenn keine bilaterale Kraniektomie durchgeführt wurde. In dieser Gruppe zeigte die T2RT einen Trend zu den niedrigsten Mittelwerten für die Behandlungsgruppe mit intaktem Schädel, verfehlte aber die statistische Signifikanz. Nichtsdestotrotz scheinen diese Daten darauf hinzudeuten, dass die Neuroprotektion der EPO-Vorbehandlung bei transientem MCAO einen starken antiödematösen Effekt bewirkt.

Wir verwendeten ein flat-panel Volumen CT für die nicht-invasive, dynamische Bildgebung der zerebralen Perfusion nach transientem Verschluss der A. cerebri media in den kortikalen und subkortikalen Regionen des Infarkts und der gesamten Hemisphäre[168]. Ohne Kraniektomie führte die EPO-Vorbehandlung zu einem signifikanten Anstieg des zerebralen Blutflusses (CBF) in den kortikalen Regionen des ischämischen Gewebes. In subkortikalen Bereichen des Infarkts und in der gesamten Hemisphäre konnten jedoch keine signifikanten Veränderungen des CBF festgestellt werden. Xiong et al. beschrieben eine EPO-Neuroprotektion nach traumatischer Hirnschädigung auch in EpoR-Null-Mäusen und führten diesen Effekt insbesondere auf einen vaskulären Schutz zurück[169]. Li et al. untersuchten die Angiogenese bei Mäusen, die 30 Minuten vor und einmal täglich nach einem ischämischen Schlaganfall rhEPO erhielten und beobachteten eine erhöhte angiogene Aktivität zwischen Tag 7 und 21. An Tag 14 erreichte der CBF die Ausgangswerte vor der Ischämie[170]. Weiterhin führte in einem Kaninchenmodell für Subarachnoidalblutungen intravenös verabreichtes rhEPO zu einem signifikant erhöhten CBF zwischen Tag 2 und 16[171]. Zusätzlich zu diesen Beobachtungen der zeitabhängigen Effekte von EPO konnten Shafi et al. an isolierten Aa. cerebri mediae von Ratten nachweisen, dass luminal appliziertes EPO die Arterien direkt dilatieren konnte und dass eine 24-stündige Vorbehandlung mit EPO diesen Effekt potenzierte[172]. Nach einer Vorbehandlung mit einer Einzeldosis EPO und transientem Verschluss der A. cerbri media beobachteten wir lediglich in den kortikalen Regionen des Infarkts und bei fehlender Kraniektomie einen signifikanten Anstieg des CBF. Wird mittels Kraniektomie der kompressive Effekt auf das Gehirn, die Mikrovaskulatur und vermutlich auch auf die pialen und venösen Gefäße aufgehoben, ist der CBF in EPO- und Placebo-behandelten Ratten gleich. Dies scheint einen lokalen Effekt für den umschriebenen Bereich der Infarktgebietes darzustellen, da keine Unterschiede im CBF für die gesamte Hemisphären, unabhängig von der EPO-Behandlung oder der Kraniektomie, vorlagen. Eine fokale Verbesserung des CBF in den kortikalen Regionen des ischämischen Areals kann auf eine effizientere Kollateralisierung bei Vorbehandlung mit EPO hinweisen, entweder über die antiödematöse und damit drucksenkende Wirkungsweise oder aufgrund direkter vasodilatativer Effekte. Die verbesserte

Kollateralisierung wiederum unterstützt die Erholung in kritisch perfundierten penumbralen Arealen, welche die Größe des Infarktkerns reduzieren, was sich durch eine signifikante Reduktion des ischämischen Läsionsvolumens gezeigt hat. In Übereinstimmung mit den oben genannten Daten zur Infarktgröße und Ödemreduktion konnte die Reduktion des Läsionsvolumen nur in Abwesenheit einer Kraniektomie beobachtet werden, d.h. in einer Situation, in der die Druckschwankungen am stärksten ausgeprägt sind.

Bei der Interpretation der Ergebnisse unserer Studie ist anzumerken, dass hier lediglich Surrogatparameter für einen sekundär neuroprotektiven Wirkmechanismus berücksichtigt wurden. Diese können als hypothesengenerierend angesehen werden, müssen aber müssen anschließend in entsprechenden „mechanistischen“ Studien bestätigt werden, um objektiv einen direkten oder indirekten Wirkmechanismus zu unterscheiden.

In dieser Studie wurde rekombinantes humanes EPO verabreicht - eine Verbindung, die aufgrund ihrer geringen BHS-Permeabilität in vergleichsweise hohen intravenösen Dosen verabreicht werden muss, was zu mehreren dosisabhängigen Nebenwirkungen führt, wie erhöhtem Hämatokrit, Bluthochdruck sowie prokoagulatorischen und prothrombotischen Effekten auf die Mikrozirkulation. Diese Nebenwirkungen scheinen in erster Linie auf die erythropoetische Wirkweise des EPO-Derivats zurückzuführen zu sein, und es ist prinzipiell denkbar, dass sie das Ausmaß der Neuroprotektion im Zusammenhang mit akuten zerebrovaskulären Erkrankungen vermindern. Daher gab es in anderen Arbeitsgruppen Bemühungen, die EPO-vermittelte Zytoprotektion zu unterstützen, ohne dass es dabei zur Beeinflussung des hämatopoetischen Systems kommt. Es konnte gezeigt werden, dass, vermutlich aufgrund einer veränderte Rezeptorinteraktion, carbamyliertes EPO und Mutanten wie EPO-S100E oder EPO-R103E neuroprotektiv wirkten, aber keine erythropoetische Aktivität aufgrund einer deutlich verminderten Affinität zum (EPOR)2-Rezeptor besaßen. Das Fusionsprotein EPO-TAT kann die BHS deutlich einfacher überwinden und ermöglicht so den Einsatz niedrigerer Dosen[173,174]. Es bleibt daher zu diskutieren, ob die Verwendung eines anderen EPO-Derivats zu deutlicheren Ergebnissen führen würde.

Ein weiterer pharmakokinetischer Aspekt von besonderem Interesse im Zusammenhang mit der Anwendung von EPO vor einer Infarktinduktion muss ebenfalls berücksichtig werden. In einem Nagetiermodell der traumatischen Hirnverletzung wurde nicht nur gezeigt, dass EPO bei peripherer Applikation hoch dosiert werden muss und dass die intravenöse Verabreichung der intraperitonealen Verabreichung überlegen ist, sondern auch, dass rhEPO die BHS erst mit einer zeitlichen Latenz von etwa 4 h passiert und seine biologische Wirkung nach etwa 8 h zu entfalten scheint[175]. Außerdem wird geschätzt, dass die Halbwertszeit von rhEPO nach einmaliger Injektion zwischen 25,6 h und 35,5 h liegt[176]. Wenn diese pharmakokinetischen Phänomene und die Ödemdynamik nach dem Hirninfarkt mit Beginn unmittelbar nach der

Ischämie berücksichtigt werden, könnte eine noch frühere Gabe von EPO möglicherweise zu einem stärker ausgeprägten neuroprotektiven Effekt führen können.

Da sich bezüglich der Effektstärken des Infarktvolumens und den Perfusionsparametern statistisch signifikante Unterschiede zeigten, scheint die Anzahl der Tiere pro Gruppe ausreichend zu sein. Dennoch bleibt es fraglich, ob größere Gruppen möglicherweise zu signifikant anderen Ergebnissen bei den Untersuchungen zum Hirnödem geführt hätten. Die klinischen Tests zeigten keine statistisch signifikante funktionelle Verbesserung, was auf eine begrenzte Sensitivität der klinischen Tests im Allgemeinen oder in Bezug auf die in dieser Studie gewählten zeitlichen Parameter und Zeitpunkte hinweisen könnte. Außerdem erlaubt das Studiendesign keine Quantifizierung einer möglichen langfristigen Verbesserung. Grundsätzlich können die Verwendung gesunder Tiere, kontrollierte Laborbedingungen und die Anwendung von Anästhesie die Übertragbarkeit der Befunde vom Labor auf die klinische Situation erschweren, was bei der Interpretation der Befunde berücksichtigt werden muss.

Unsere Studie zeigte, dass eine einmalige Gabe von 5.000IU/kg rhEPO vor einem transienten Verschluss der A. cerebri media bei Ratten das Volumen der ischämischen Läsion signifikant verringert, das Ausmaß der Mittellinienverlagerung reduziert und den lokalen CBF in den kortikalen Ischämie-Regionen nach 24 Stunden erhöht. Die Daten könnten auf eine Interaktion zwischen ödem- und druckreduzierenden sowie durchblutungssteigernden Effekten, die durch EPO vermittelt werden, hindeuten.

3.2. Klinische Aspekte

3.2.1. Validierung der deutschen Übersetzung der FOIS

Die FOIS wird sowohl in Klinik als auch in Forschung als ein sehr valides und reliables Instrument zur Beurteilung der funktionellen oralen Ernährung eingesetzt. In Ermangelung einer validen deutschen Version dieser Skala und im Bestreben, unserer Studien nach den Richtlinien der Good Clinical Practice durchzuführen, war das Ziel unserer Studie die Validierung und die Übersetzung der FOIS-Skala ins Deutsche (FOIS-G).

Wir führten die Übersetzung mit Hilfe des Teamansatzes der TRAPD-Methodik (Translation, Review, Adjudication, Pretest, Documentation) ohne den Zwischenschritt der Rückübersetzung durch. Obwohl die Rückübersetzung üblicherweise bei Anpassungsprozessen von beispielsweise Fragebögen und Erfassungsbögen angewandt wird[177,178], hat die Methode der Rückübersetzung keinen wissenschaftlich fundierten Hintergrund und führt auch nicht zwangsläufig zu einer höheren Qualität der Endversion[179,180].

Der Validierungsprozess orientierte sich am Studiendesign der Originalskala. Aufgrund von Unterschieden in der Umsetzung von Schlaganfall-Outcomeparameter wurden nicht alle in der Originalarbeit verwendeten Items in die Validierung der deutschen Version aufgenommen. Die Inter-Rater-Reliabilität war sowohl für die Vortestung durch zwei erfahrene Logopäden als auch für die Bewertung durch sechs erfahrene Logopäden hoch und zeigte eine signifikante Korrelation zwischen allen einbezogenen Schlaganfallqualitätsmarkern (NIHSS-Score, modifizierte Rankin Skala [mRS] und BI) und dem FOIS-G. Eine Diskrepanz zwischen den beiden Logopäden im Rahmen der Vortestung konnte nach Rücksprache mit dem ursprünglichen Autor der Skala geklärt werden.

Wie erwartet fanden sich signifikante Korrelationen zwischen dem FOIS-G und allen Outcome-Parametern. Diese Ergebnisse sind trotz nicht ganz gleicher Studiendesigns der ursprünglichen Arbeit sehr ähnlich. Die FOIS-G Inter-Rater-Reliabilität mit K = 0,96 für die Vortestung sowie die prozentuale Übereinstimmung für alle sechs gepaarten Bewerter sind mit 81% bis 94% für FOIS-G vs. 85% bis 95% für den ursprünglichen FOIS als hoch zu werten. Die Spearman-Rangkorrelation zwischen allen Bewertern in FOIS-G beträgt r_s = 0,96 bis r_s = 0,99 (original FOIS r_s = 0,98 bis r_s = 0,99). Die Gesamtübereinstimmung zwischen allen sechs gepaarten Ratern für FOIS-G beträgt K = 0,83 (ursprünglicher FOIS K = 0,86 bis K = 0,91).

Hinsichtlich der Kriteriumsvalidität ohne Dichotomisierung korrelierten die Schlaganfallqualitätsmaße (mRS, BI und 70 ml-Wassertest) signifikant mit dem FOIS-G sowohl in der Vortestung als auch in der Auswertung durch die sechs Rater für die Werte vor dem Schlaganfallereignis, bei Aufnahme in die Klinik und bei der Entlassung aus der Stroke

Unit. Wie auch in der Originalarbeit zeigten unsere dichotomisierten Daten für die mRS, den BI und den 70 ml-Wassertest eine signifikante Assoziation zwischen FOIS-G und mRS bei Entlassung sowohl bei der Vortestung als auch bei den Durchschnittswerten der 6 Bewerter. Äquivalent zu den Ergebnissen der Kreuzvalidierung der ursprünglichen FOIS und der VFSS lag eine signifikante Korrelation zwischen FOIS-G und dem PAS-Score (Penetrations-Aspirations-Skala nach Rosenbek) in der FEES in der Vortestung und auch in den durchschnittlichen Werten der 6 Bewerter. Der 70ml-Wassertest in einer Unterstichprobe von 76 Probanden zeigte eine signifikante Korrelation mit der FOIS-G.

Zum Zeitpunkt der Durchführung unserer FOIS-G Übersetzung- und Validierungsstudie existieren eine chinesiche und eine italienische validierte Übersetzung der FOIS. Obwohl die Adaptationen der chinesischen und der italienischen Version der FOIS in unterschiedlichen Studiensettings durchgeführt wurden, finden sich auch bei diesen Ergebnissen sehr starke Übereinstimmungen: Die Inter-Rater-Reliabilität ist sowohl bei der chinesischen wie auch der italienischen Version hoch (italienische FOIS ICC = 0,99; chinesischer FOIS K = 0,881, Spearman Rangkorrelation r_s = 0,972; chinesischer Wasserschlucktest K = 0,844, Spearman Rangkorrelation r_s = 0,965). Für die italienische FOIS-Version wurden weder die Berechnung der Kriteriumsvalidität noch die Kreuzvalidierung durchgeführt. Die Ergebnisse der chinesischen Version sind der originalen FOIS und der FOIS-G sehr ähnlich, wobei eine starke Korrelation zwischen FOIS und dem Wasserschlucktest gefunden wurde. Außerdem sind NIHSS und BI ebenfalls signifikant mit der chinesischen FOIS assoziiert. Die Kreuzvalidierung zeigte eine hohe Assoziation mit der chinesischen FOIS und dem Vorhandensein von Dysphagie und Aspiration in der VFSS.

Trotz der unterschiedlichen Ansätze der Adaptationen der originalen FOIS-Skala ist erkennbar, dass alle drei validierten Übersetzungen eine hohe Inter-Rater-Reliabilität aufweisen und, mit Ausnahme der italienischen Version, sehr starke Korrelationen für Kriteriumsvalidität und Kreuzvalidierung zeigen.

Die unterschiedlichen Studiendesigns sind unter anderem der Tatsache geschuldet, dass weltweit keine einheitlichen Leitlinien für das Dysphagiemanagement existieren. Sowohl die verwendeten Screeningtests als auch die Wahl der bildgebenden Diagnostik (FEES oder VDSS) variieren alleine in Deutschland von Klinik zu Klinik.

Die Ähnlichkeit in der Inter-Rater-Reliabilität zwischen allen drei übersetzten Versionen der ursprünglichen FOIS ist auf die gute Konsens- und Kriteriumsvalidität der ursprünglichen FOIS-Skala zurückzuführen. Die ähnlichen Ergebnisse für die Kreuzvalidierung in allen drei validierten Übersetzungen deuten auf eine hohe Validität der FOIS sowohl für die VFSS als auch für die FEES hin.

In Deutschland hat sich die FEES zum Goldstandard der instrumentellen Dysphagiediagnostik entwickelt und wird in mehr als 70% der Stroke Units eingesetzt, während die VFSS nur in wenigen Einrichtungen des Landes zu finden ist[63]. Die Validierung der FOIS-G für die FEES steigert sowohl den Wert der FOIS als auch der FEES-Untersuchung in der Forschung und im klinischen Umfeld.

In der transnationalen klinischen Patientenversorgung wie auch in der Forschung stellt die Verwendung von Scores eine Möglichkeit dar, eine internationale Vergleichbarkeit zu ermöglichen und herzustellen. In diesem Zusammenhang ist jedoch eine gründliche Validierung jedes Tests in der jeweiligen Landessprache eine unabdingbare Voraussetzung. Im Fall der FOIS ist die vorliegende deutsche Version erst die dritte Übersetzung (neben der italienischen und chinesischen Version[181,182]) von der 2005 in englischer Sprache veröffentlichten Originalskala. Dieser Umstand unterstreicht zum einen den Bedarf an zusätzlichen Übersetzungen und Validierungen und andererseits die Notwendigkeit für die gleichzeitige Entwicklung neuer Skalen in einer Vielzahl von Sprachen.

Das Design und die Ergebnisse der vorliegenden Studie sowie der Vergleich mit bestehenden Adaptationen zeigen die Notwendigkeit eines weltweit einheitlichen Ansatzes bei der Gestaltung des Dysphagiemanagements. Die Verwendung von validierten Skalen in mehreren Sprachen ist ein wichtiger Schritt in diese Richtung.

Zusammenfassend wurde die FOIS-G nach internationalen Übersetzungsrichtlinien übersetzt und von erfahrenen Logopäden mit Deutsch als Muttersprache validiert. Sie stellt ein valides Instrument für die Bewertung der funktionellen oralen Aufnahme von Flüssigkeiten und Nahrung bei Dysphagiepatienten dar und wurde in den beiden folgenden Studien angewandt.

3.2.2. FEES zur Einschätzung der Ernährungsweise bei Patienten mit akutem Schlaganfall

Dysphagien sind eine häufige Störung bei Schlaganfallpatienten und eine dysphagiebedingte Aspirationspneumonie stellt den häufigsten Grund für ein schlechtes Behandlungsergebnis mit Behinderung oder Versterben des Patienten dar. Durch ein klinisches Dysphagiemanagement kann definitionsgemäß eine stille Penetration oder Aspiration nicht festgestellt werden. Unsere Studie untersuchte mittles FEES, inwiefern eine an die Ressourcen des Patienten angepasste Ernährungsform gewählt wurde. Auch wurde untersucht, ob durch eine entsprechende Anpassung der Ernährungsform eine Reduktion potentieller Sekundärkomplikationen resultiert.

In unserer Studie wurden insgesamt 173 FEES-Untersuchungen bei 152 Patienten durchgeführt. Relevante Komplikationen durch die FEES, wie ein Laryngospasmus, Synkopen, oder behandlungsbedürftiges Nasenbluten wurden nicht beobachtet. Damit gehen wir von einer hohen Sicherheit für den Patienten bei der Durchführung der Untersuchung aus.

Eine relevante Dysphagie konnten wir mittels FEES bei 72% unserer Schlaganfallpatienten feststellen. Lediglich 30,9% der Patienten hatten nach klinischer Schluckuntersuchung eine für ihre Schluckfunktion adäquate Ernährung. Im Umkehrschluss musste also bei mehr als zwei Dritteln der Patienten eine Umstellung der Ernährung (gemessen an einer Änderung des FOIS-Scores) nach instrumenteller Diagnostik erfolgen, was ein alarmierendes Ergebnis auf einer eigentlich spezialisierten Schlaganfallbehandlungseinheit darstellt. Dies spricht zum einen für ein immer noch nur geringes Bewusstsein für das Vorhandensein von Schluckstörungen und zum anderen für die deutlichen Einschränkungen von Screening-Untersuchungen (in unserer Klinik wurde zum Zeitpunkt der Studie der GUSS [Gugging Swallowing Screen] als Test[183] verwendet) sowie klinisch-logopädischer Diagnostik.

Dysphagische Patienten hatten bei der Aufnahme und bei der Entlassung einen höheren NIHSS und mRS. Dies bestätigt die Ergebnisse von Dziewas et. al, die zeigten, dass Patienten mit einem NIHSS > 3 Penetrationen und Aspirationen aufwiesen[118]. Warnecke et al. resümierten, dass der Schweregrad der Dysphagie prädiktiv für das funktionelle Ergebnis drei Monate nach dem ersten Schlaganfall ist[50]. Somit scheint der Schweregrad funktioneller Defizite prädiktiv für eine Dysphagie zu sein und umgekehrt.

Eine Dysphagie wurde häufiger bei rechtshemisphärischer Ischämie diagnostiziert. Teismann et al. konnten mittels Magnetenzephalographie eine zeitabhängige kortikale Aktivierung der Hemisphären während des Schluckens nachweisen. Während es in der oralen Phase des Schluckens zu einer überwiegend linkshemisphärischen Aktivierung kam, wurde während der

pharyngealen Phase eine rechtshirnige Aktivierung beobachtet[184]. Da wir die Penetration und Aspiration, die während der pharyngealen Phase des Schluckens auftreten, häufiger bei unseren Patienten mit rechtshemisphärischer Ischämie beobachteten, stimmen unsere Daten mit diesen Befunden überein. Rechtsseitige Hirnläsionen sind auch mit einem Neglect und einem verminderten Bewusstsein für Störungen (Anosognosie) assoziiert, was die Patienten zur Aspiration prädisponiert[185,186]. Diese könnte eine zusätzliche Erklärung für unsere Befunde sein.

Wurde die Ernährung auf Grundlage der Untersuchungsergebnisse in der FEES angepasst, beobachteten wir ein besseres funktionelles Outcome bei Entlassung, eine geringere Notwendigkeit für eine Intubation, eine niedrigere Pneumonie-Rate, eine geringere Sterblichkeit und eine kürzere Dauer des Krankenhausaufenthalts. In einem Review von Steele et al. wurde eine signifikante Reduktion von Penetration und Aspiration nach dem Andicken von Flüssigkeiten beschrieben[64]. Dies könnte ein Faktor sein, der unsere Ergebnisse erklärt. Ein weiterer Faktor, der zu einem besseren Ergebnis beitragen könnte, ist die erhöhte Mobilität der Patienten nach der Entfernung einer nasogastralen Ernährungssonde oder einer intravenösen Kanüle zur parenteralen Ernährung, was die Wirkung der Physiotherapie verstärkt. Bei einer Anpassung der oralen Ernährung wurde das Risiko einer Pneumonie oder Intubation reduziert.

Unsere Ergebnisse unterstreichen den Wert der FEES, um eine sichere orale Ernährung für Schlaganfallpatienten zu ermöglichen. Wir befürworten daher den niederschwelligen Einsatz der FEES bei Schlaganfallpatienten, auch im Sinne einer primären Screening-Untersuchung mittels FEES. Die Untersuchung kann jederzeit schnell, komplikationsarm und mit allenfalls mäßiger Belastung für die Patienten durchgeführt werden. Insbesondere können mit Hilfe der FEES stille Penetrationen und Aspirationen festgestellt, die adäquate Ernährung gewählt und gegebenenfalls störungsbasierte logopädische Übungen geplant werden. Eine frühe Durchführung der FEES auf der Stroke Unit ist daher nach unserer Einschätzung zu empfehlen. Auch andere Autoren empfahlen bereits eine primäre Dysphagiediagnostik beim Schlaganfall mittels FEES[187], allerdings basierten diese Empfehlungen rein auf der Überlegung, dass „stille Veränderungen“ ohne instrumentelle Diagnostik nicht erfasst werden können.

Kürzlich wurde eine große FEES-Registerstudie mit verschiedenen neurologischen Erkrankungen veröffentlicht. Die Ergebnisse dieser Studie bestätigen ebenfalls die Sicherheit der FEES, auch wenn sie von einem unerfahrenen Untersucher durchgeführt wird. In dieser Studie wurde die Ernährung bei etwa 50% der Patienten nach der FEES-Untersuchung angepasst[188].

Bisher wurde lediglich eine Studie über den Einfluss von FEES auf das funktionelle Ergebnis bei Schlaganfallpatienten veröffentlicht[189]. Bax et al. fanden eine Reduktion der Pneumonie-Rate und eine höhere Rate an normaler Ernährung bei Entlassung nach FEES im Vergleich zu dem Zeitraum, als noch keine FEES in ihrer Klinik durchgeführt wurde. Die Patienten in dieser Studie hatten eine längere Krankenhausverweildauer als in als in unserer Studie und es gab keine Unterschiede in der Mortalität wenn die FEES routinemäßig angewandt wurde. Allerdings hat diese Studie potenzielle Schwächen: (i) Die Autoren verglichen ihre Patienten mit einer historischen Kontrollgruppe; (ii) in beiden Gruppen wurde die Mehrheit der Patienten nicht mittels FEES untersucht; und (iii) in Bezug auf die funktionellen Ergebnisse wurden die Scores der häufig verwendeten NIHSS und mRS bei Entlassung nicht berichtet. Daraus folgend können keine Rückschlüsse bezüglich des Einflusses der FEES auf das neurologisch-funktionelle Ergebnis gezogen werden.

Zusammenfassend zeigte unsere Studie Assoziationen zwischen der Anpassung der Ernährung auf Basis der FEES-Befunde und dem funktionellen neurologischen Outcome, der Notwendigkeit zur Intubation und der Rate an Pneumonien und Mortalität.

Die FEES kann Risikopatienten mit akutem Schlaganfall besser identifizieren als das Screening auf Dysphagie oder eine klinisch-logopädische Untersuchung, da sie in der Lage ist, stille Aspiration zu erkennen. Sie ist ein sicheres und schnelles Verfahren, das bei etwa zwei von drei Patienten zu einer Anpassung der oralen Ernährung führt, mit potenziell positiven Folgen für das klinische Gesamtergebnis durch die Vermeidung von Pneumonien oder mechanischer Beatmung. Auch kann anhand des Pathomechanismus der Dysphagie eine störungsgeleitete logopädische Therapie erfolgen. Basierend auf unseren Daten, und trotz der Notwendigkeit groß angelegter und randomisiert-kontrollierter Studien, empfehlen wir den niederschwelligen Einsatz der FEES.

3.2.3. FEES zur Einschätzung der Ernährung bei neurologischen Intensivpatienten

Nicht nur für Schlaganfallpatienten ist die Pneumonie als Folge einer Dysphagie eine große Gefahr. Insbesondere für kritisch kranke, intensivmedizinisch behandelte Patienten stellt die Pneumonie einen der stärksten Prädiktoren für eine erhöhte Sterblichkeit dar[190,191]. Daher untersuchten wir auch auf unserer neurologischen Intensivstation, ob die Patienten eine fähigkeitsangepasste Ernährung hatten und ob eine Anpassung der Ernährung zu weniger Komplikationen führte. Da das Kollektiv der Schlaganfallpatienten den größten Teil der Patienten auf dieser Station darstellte, erfolgte eine entsprechende Subgruppenanalyse.

In dieser Studie wurde die FEES insgesamt 144-mal bei 125 Intensivpatienten durchgeführt. Eine Dysphagie zeigte sich in diesem Kollektiv bei 72% der Patienten.

Insgesamt wurde bei 64% der Patienten eine Anpassung der Ernährung empfohlen. Wie auch in der Studie zuvor zeigte sich also, dass trotz einer hohen Spezialisierung des Behandlungsteams nach klinischer Schluckuntersuchung nur knapp ein Drittel der Patienten eine ihren Fähigkeiten angepasste Ernährung hatten. Bei etwa einem Drittel dieser Patienten wurde mit Hilfe der FEES eine stille Aspiration festgestellt. Auch im Kollektiv der neurologischen Intensivpatienten zeigt sich, dass mit Hilfe von Screening-Tools oder einer klinisch-logopädischen Untersuchung die adäquate Ernährungsweise sich nicht sicher einschätzen lässt. Dies führte entweder zu einer zu restriktiven Einschränkung der Ernährung mit der Gefahr der Mangelernährung und Einschränkung der Lebensqualität der Patienten oder ging umgekehrt mit einer möglichen Gefährdung der Patienten einher. Frühere Studien konnten zeigen, dass Dysphagien, die nicht die orale Phase betreffen, bei rein klinisch-logopädischer Untersuchung der Patienten zu einer restriktiveren Einschränkung der Ernährung als nötig führen[192]. Weitere Studien zeigten ebenfalls eine reduzierte Sensitivität und Spezifität bei nicht-instrumenteller Dysphagiediagsnotik[193-195].

In der Subgruppe der intensivmedizinisch behandelten Schlaganfallpatienten zeigte sich bei den Patienten mit einer Dysphagie sowohl bei Aufnahme als auch bei Entlassung ein größeres funktionelles Defizit als bei Patienten ohne Dysphagie. Dies bestätigt nochmals in diesem Kollektiv, dass ein schwerwiegenderes neurologisch-funktionelles Defizit prädiktiv für eine Dysphagie ist und umgekehrt[118]. Auch bestätigt sich hier das schlechtere funktionelle Behandlungsergebnis[50], wie auch in der vorangegangenen Studie. Bei den Schlaganfallpatienten mit Dysphagie war häufiger eine Intubation und maschinelle Beatmung notwendig. Dies deckt sich ebenfalls mit den Ergebnissen anderer Studien. Die Dysphagie führt bei den Schlaganfallpatienten zur Pneumonie, was die Intubation und maschinelle Beatmung zur Folge haben kann. Intubation und Beatmung sind jedoch wiederum

Risikofaktoren für die Entwicklung einer Dysphagie, beispielsweise durch Veränderungen der oropharyngealen Sensibilität oder durch muskuläre Schwächen im Rahmen einer zusätzlich sich entwickelnden Critical-Illness-Polyneuro-Myopathie[119,196,197]. Da die Schlaganfallpatienten lediglich mittels eines Screening-Tests untersucht wurden (GUSS[183]), hätte möglicherweise eine eingehende klinisch-logopädische Untersuchung zu einer besseren Einschätzung der Ernährungsstrategie geführt. Es lagen jedoch keine wesentlichen Unterschiede zwischen den mittels GUSS untersuchten Schlaganfallpatienten und den rein klinisch beurteilten Patienten ohne Schlaganfall vor. Wir würden daher bei anderer Vorgehensweise keine wesentlichen Unterschiede erwarten. Darüber ist die Reihenfolge Screening-Test, logopädische Untersuchung und dann FEES, zumindest auf unserer Intensivstation, nicht die übliche klinische Routine bei Schlaganfallpatienten.

Patienten, die nach FEES auf NPO gesetzt wurden, hatten eine höhere Sterblichkeit, mussten häufiger intubiert und tracheotomiert werden im Vergleich zu Patienten, die zumindest eine geringe Menge an oraler Ernährung oder Flüssigkeitszufuhr erhielten. Kritisch kranke Patienten auf einer Intensivstation werden tendenziell unterernährt, was sie für einen Abbau an Muskelmasse prädisponiert und wodurch die Auswirkungen einer zusätzlich auftretenden Critical-illness-Polyneuro- und Myopathie noch verstärkt werden können[47,198]. Dies führt zu einer weiteren Verschlechterung der Schluckfunktion, was mit einer Aspiration und einer konsekutiven Pneumonie einhergehen kann mit den Folgen einer erhöhten Mortalität[119].

Unsere Ergebnisse unterstreichen die Notwendigkeit einer instrumentellen Diagnostik bei neurologischen Patienten, die auf einer Intensivstation behandelt werden. Unserer Meinung nach sollte die Indikation zur FEES niederschwellig angesetzt werden, insbesondere in der Hochrisikogruppe der neurologischen Intensivpatienten. Mittlerweile wird bei nahezu allen Patienten unserer Intensivstation eine FEES durchgeführt. Die FEES stellt auch bei Intensivpatienten ein sicheres, schnelles und zuverlässiges Untersuchungsinstrument dar. Wir beobachteten keine schwerwiegenden Nebenwirkungen. Nach unserer Meinung sollte die FEES bei Patienten auf der neurologischen Intensivstation frühzeitig durchgeführt werden, unabhängig von einem Screening oder einer klinisch-logopädischen Untersuchung. Letztere ist dennoch ergänzend notwendig, um den Pathomechanismus der Dysphagie zu formulieren, damit entsprechende logopädische Übungen ausgewählt werden können.

Auch andere Studien haben bereits den Einsatz von FEES auf der Intensivstation empfohlen, aber diese Studien hatten nicht einen Fokus auf neurologischen Patienten[126,125]. Die Studie von Hafner et al. berichtet über den Einsatz von FEES bei 553 Patienten mit unterschiedlichen Krankheitsentitäten über einen Zeitraum von 45 Monaten. Zwar wurden das Vorliegen einer Dysphagie und von Kostformanpassungen berichtet, jedoch nicht die Pneumonierate, der funktionelle Behinderungsgrad sowie die Mortalität[126].

Die Dysphagie hatte bei unseren Patienten keinen Einfluss auf die Pneumonierate. Unsere Daten sind sicherlich dadurch verzerrt, dass die schwerkranken Patienten, präklinisch aspirierten und in den ersten Tagen der Behandlung eine Pneumonie entwickelten, da unser Studiendesign nicht den Zeitpunkt des Auftretens der Pneumonie differenzierte.

Verglichen mit einem Screening-Test oder einer klinischen Beurteilung kann die FEES als eine überlegene Methode zur Erkennung von Penetration und Aspiration bei neurologischen Intensivpatienten angesehen werden. Dies wird auch dadurch gestützt, dass die Mehrheit der Patienten eine Ernährung hatte, die nicht ihren Schluckfähigkeiten entsprach. Bei mehr als einem Viertel unserer Patienten wurde eine stille Aspiration nicht durch klinische Beurteilung festgestellt. Diese Erkenntnis ist von äußerster Wichtigkeit, da die Vermeidung von dysphagie-bedingten Komplikationen einen Einfluss auf die Mortalität und Morbidität in dieser Population haben könnte. Da die FEES mit einem geringen periprozeduralen Risiko durchgeführt werden kann, empfehlen wir eine breite und frühzeitige Anwendung der FEES bei allen neurologischen Intensivpatienten im Sinne eines Screenings auf Dysphagien, zur Auswahl einer adäquaten Ernährung und zur individuell störungsspezifischen logopädischen Übungsauswahl für die Patienten.

3.2.4. Pharnygeale Elektrostimulation bei tracheotomierten Schlaganfallpatienten

Während der Behandlung auf einer Intensivstation ist bei Schlaganfallpatienten häufig eine Tracheotomie aufgrund einer Dysphagie notwendig, was mit höherer Pflegebedürftigkeit, höheren Behandlungskosten und deutlichen Einschränkungen der Lebensqualität einhergeht. Aufgrund einer fortbestehenden Dysphagie ist häufig eine Dekanülierung nicht möglich. Daher untersuchte die multizentrische PHAST-TRAC-Studie, ob durch Anwendung der PES eine frühere Dekanülierung erreicht werden kann.

Bei Anwendung der PES wurden in dieser Studie 17 (49 %) von 35 Patienten als dekanülierungsfähig von den Untersuchern eingestuft, während lediglich 3 (9 %) von 34 Patienten in der Scheinstimulationsgruppe als dekanülierungsfähig eingestuft wurden. Das Ansprechen auf die Behandlung schien mit einer kürzeren Zeit vom Beginn der Schlaganfallsymptomatik bis zur Randomisierung und einer kürzeren Dauer der mechanischen Beatmung zusammenzuhängen. Sowohl nach dem randomisierten als auch nach dem offenen Teil der Studie waren 37 (57%) von 65 Patienten, die eine PES erhielten, bereit für eine Dekanülierung und in einer Posthoc-Analyse wurden Patienten, die auf die PES ansprachen, signifikant früher aus dem Krankenhaus entlassen als nicht-Ansprechende.

Die Effektstärke der PES in dieser Studie entsprach einer früheren monozentrischen Pilotstudie[138]. Auch zeigte sich eine ähnlich niedrige Spontanheilungsrate in der Scheinstimulationsgruppe wie in der DECAST-Studie, einer Kohortenstudie, in der nur 14 (26%) von 53 Schlaganfallpatienten, die eine Tracheotomie hatten, innerhalb von 3 Monaten nach dem Schlaganfall dekanüliert werden konnten[136].

Der Zusammenhang zwischen der Behandlungseffizienz und der kurzen Zeit bis zum Beginn der Behandlung steht vermutlich auch im Zusammenhang mit der Entwicklung einer Critical-Illness-Dysphagie aufgrund einer Critical-Illness-Polyneuro-Myopathie (CIP/CIM) bei Patienten mit längerer Behandlung auf der Intensivstation und mechanischer Beatmung[119,199]. Neben der schlaganfallbedingten Beeinträchtigung des zentralen Schlucknetzwerks schädigen CIP und CIM schluckrelevante Hirnnerven bzw. Muskeln. Außerdem ist die PES von der Intaktheit laryngealer und pharyngealer sensibler Bahnen abhängig, sodass eine schwere Polyneuropathie die postulierte Wirkung auf die Plastizität des Gehirns beeinträchtigen könnte. Obwohl die vorliegende Studie keine neurophysiologischen Untersuchungen umfasste, unterstützen die höheren sensiblen Schwellenwerte, die bei Patienten beobachtet wurden, die nicht für eine Dekanülierung bereit waren, im Vergleich zu den erfolgreich behandelten Patienten, die Vorstellung einer gestörten sensorischen Rückkopplung als wichtiger Grund für ein Scheitern der Behandlung.

Ein weiteres wichtiges Ergebnis dieser Studie war, dass sich ein zweiter Zyklus der PES bei vier (27 %) von 15 Patienten als wirksam erwies, die nach einem ersten Behandlungszyklus mit der PES noch nicht dekanüliert werden konnten. Dieses Ergebnis steht im Einklang mit einer offenen Kohortenstudie[200] und legt nahe, dass Patienten, die auf einen Behandlungszyklus (3 Tage) nicht ansprechen, erneut behandelt werden sollten.

Die positiven Ergebnisse der vorliegenden Studie unterscheiden sich deutlich von den Ergebnissen der STEPS-Studie, in der dysphagische Schlaganfallpatienten ohne Tracheotomie oder beatmet mit PES behandelt wurden[77]. In dieser Studie wurde die PES nach dem gleichen Behandlungsparadigma durchgeführt, jedoch zeigte sich keine Überlegenheit gegenüber der Scheinstimulation in Bezug auf die Schlucksicherheit (primärer Endpunkt) oder klinischen Dysphagie-Scores (sekundäre Endpunkte) in einer Kohorte von 162 Schlaganfallpatienten. Obwohl keine eindeutigen Schlussfolgerungen in Bezug auf Gründe für diese diskrepanten Ergebnisse gezogen werden können, könnten Unterschiede zwischen den Studien einige Erklärungsansätze liefern. Erstens: Die STEPS-Studie rekrutierte Schlaganfallpatienten, die eine geringere Schwere des Schlaganfalls aufwiesen (mittlerer NIHSS <10 in STEPS vs. 17,5 in PHAST-TRAC). Außerdem hatten viele der Patienten bereits zumindest eine teil-orale Ernährung bei Studieneinschluss. Zweitens betrug in der STEPS-Studie die mediane Zeit zwischen Beginn der Behandlung und Randomisierung 11 Tage im Vergleich zu 28 Tagen bei PHAST-TRAC, was darauf hindeuten könnte, dass die Spontanerholungen der Schluckfunktion in STEPS möglicherweise häufiger auftraten als in der PHAST-TRAC-Studie. Auch waren die verwendeten Stimulationsintensitäten unterschiedlich, mit einem Mittelwert von 14,5 mA in STEPS verglichen mit 33,6 mA in PHAST-TRAC, was darauf schließen lässt, dass die PES in PHAST-TRAC mit einer effektiveren Dosis verabreicht wurde.

Die Stärke dieser Phase-3-Studie ist das multizentrische, scheinkontrollierte Design der Studie mit gut definierten Gruppen von Schlaganfall-Patienten und konsistenten Ergebnissen in den verblindeten und offenen Teilen der Studie. Allerdings sind einige Einschränkungen erkennbar. Erstens war die Studie zwar die größte Studie zu PES bei tracheotomierten Patienten, hatte aber eine nur geringe Zahl an Patienten, was auf das adaptive Design zurückzuführen ist, das zu einer Reduzierung der Stichprobengröße führte. Dennoch sind die Ergebnisse dieser Studie konsistent mit den anderen Ergebnissen aus dieser Studie und denen der Pilotstudie. Zweitens: Die PES wurde einfach verblindet verabreicht, wobei die Person, die die PES durchführte, nicht verblindet war. Da sowohl die Behandlung als auch die Beurteilung des Endpunktes vor Ort durchgeführt wurden, könnte es hierdurch zu Verzerrungen kommen, obwohl der Beurteiler des Endpunktes eigentlich für die Gruppenzuweisung der Patienten verblindet war. Um dieses Problem prospektiv anzugehen, wertete ein unabhängiges FEES-Prüfgremium, das für den Rekrutierungsort und die Gruppenzuweisung maskiert war, die

Videos der FEES-Untersuchung erneut aus. Das Gremium kam zu den gleichen Ergebnissen wie Beurteiler vor Ort. Drittens wurde der primäre Endpunkt einige Tage nach Ende der Behandlung bewertet. Danach wurde allen Patienten, die den primären Endpunkt nicht erreichten, unabhängig von ihrer ursprünglichen Behandlungsgruppe eine Open-Label-Behandlung angeboten. Infolgedessen konnte der langfristige Effekt der einmaligen randomisierten Behandlung auf die Ergebnisse nicht bewertet werden. Dennoch profitierten 20 Patienten von dieser zusätzlichen Möglichkeit der aktiven Behandlung. Viertens war das Durchschnittsalter der Scheinstimulationsgruppe numerisch höher als das der Behandlungsgruppe, was einen negativen Effekt auf die Heilungsrate in der Scheinstimulationsgruppe gehabt haben könnte. Da die Subgruppenanalyse jedoch keine Interaktion zwischen Alter und PES zeigte, ist es unwahrscheinlich, dass dieser Unterschied das Ergebnis beeinflusst hat. Zuletzt wurde der Großteil der Patienten von nur einem Standort rekrutiert. Die Erfahrung und der Behandlungsstandard an diesem Standort könnten das Ergebnis des primären Endpunktes beeinflussen. Daher wäre es wünschenswert, wenn die Ergebnisse der Studie nochmals in einer größeren Studie bestätigt würden. Allerdings schien die frühe Rekrutierung nach dem Schlaganfall und nicht der Rekrutierungsort das Ansprechen in multiplen Variablen-Modellen vorherzusagen.

Zusammenfassend lässt sich sagen, dass bei Patienten mit schwerer schlaganfallbedingter Dysphagie, die eine Tracheotomie benötigten, die PES sicher durchgeführt werden konnte und einer Scheinstimulation überlegen war, was zu einer Verbesserung der Schluckfunktion und einem besseren Schutz des Luftwegs führte und eine höhere Rate an Dekanülierung zur Folge hatte.

4. Zusammenfassung und Ausblick

Trotz beeindruckender Fortschritte in den letzten Jahren in der Akutbehandlung des Schlaganfalls durch die Einführung der Thrombektomie kommt diese Methode nur bei einem umschriebenen Kollektiv von Schlaganfallpatienten zur Anwendung. Die systemische Thrombolyse und die Kraniektomie kommen ebenfalls nur vergleichsweise selten zur Anwendung. Die Stroke Unit Therapie und die frühe Gabe von Acetylsalicylsäure sind zwar wirksam, jedoch ist ihr Nutzen begrenzt. Für das Groß der Patienten fehlen uns weiterhin evidenzbasierte Behandlungsstrategien, die nicht nur die Mortalität reduzieren, sondern auch den Grad der Behinderung und die Lebensqualität verbessern bzw. erhalten. Daher kommt der Suche nach neuen oder besseren Behandlungsmethoden für die Akut- oder Postakutphase der Erkrankung eine hohe Bedeutung zu.

Unser Experiment zur Sonothrombolyse unterstreicht die Wirksamkeit und Sicherheit von mmSTL in experimentellen in vivo-Schlaganfallmodellen. Dennoch zeigte eine Meta-Analyse der Sonothrombolyse mit oder ohne Verwendung von Mikrobläschen in Studien zum Schlaganfall beim Menschen keine positiven Effekte[201-203]. In diesen Studien wurden unterschiedliche Kontrastmittel wie Levovist®, Sonovue® oder Mikrosphären verwendet. In der insgesamt größten Studie zur Sonothrombolyse beim Menschen, der CLOTBUST-ER-Studie, wurde kein Kontrastverstärker eingesetzt[204]. Die Anwendung von mmSTL wird beim Menschen dadurch eingeschränkt, dass das Mikrobläschenpräparat entweder kontinuierlich oder mit mehreren Boli im Verlauf der Therapie gegeben werden müssen. Die Relevanz der Sonothrombolyse in einem klinischen Setting bleibt fraglich, insbesondere da die Studien zur endovaskulären Therapie deren große Behandlungseffekte gezeigt haben. Wie Alexandrov et al. anmerkten, werden neue Studien, die neue nicht-endovaskuläre Ansätze testen, in Zukunft nur schwer zu realisieren sein, und es ist fraglich, ob neue Studien zur Sonothrombolyse entstehen werden[205]. Die neueste Studie, die TRUST-Studie, die eine anwenderunabhängige Kopfhalterung für die Beschallung verwendet, rekrutiert bereits seit 2018 (Quelle: clinicaltrials.gov/ct2/show/NCT03519737), aber es wurden noch keine Ergebnisse veröffentlicht. Es bleibt fraglich, ob die Sonothrombolyse bei zerebralen Gefäßverschlüssen großer Gefäße eine Rolle spielen wird, da diese Patienten einer endovaskulären Therapie zugeführt werden sollten. Ein Szenario, in dem ein Patient für diese Therapie nicht in Frage käme, scheint nicht realistisch und allenfalls konstruiert zu sein. Allerdings könnte die Sonothrombolyse während des Transports von einem peripheren Schlaganfallzentrum in ein Thrombektomiezentrum eingesetzt werden. Dieses Setting wurde in der TRUST-Studie untersucht. Möglicherweise könnten Patienten mit weiter distal gelegenem Gefäßverschluss (bspw. M3/M4 Niveau), die der Thrombektomie üblicherweise nicht zugänglich sind, von der Wirkung der Sonothrombolyse profitieren. Dennoch könnte die Sonothrombolyse das

Schicksal der Neuroprotektion teilen, da sie zwar in Tiermodellen wirksam ist, aber keinen Effekt auf das funktionelle Ergebnis von Schlaganfallpatienten zeigt. Daher wird die Sonothrombolyse in der klinischen Routine noch keine Rolle spielen.

Unsere Untersuchungen zum Effekt einer EPO-Gabe beim ischämischen Schlaganfall zeigten Hinweise auf einen sekundären neuroprotektiven Effekt durch Verminderung des vasogenen Hirnödems[206]. Einschränkend ist jedoch dabei anzumerken, dass in diesem experimentellen Modell eine Vorbehandlung mit EPO erfolgte, also die Substanz vor der Induktion des Schlaganfalls verabreicht wurde. Damit lässt sich die Therapie nicht direkt auf die klinische Situation beim akuten ischämischen Schlaganfall übertragen. Wie diskutiert scheint EPO jedoch ohnehin erst verzögert seine pharmakologische Wirkung entfalten. Die nach dem Infarkt eintretende erhöhte Permeabilität der Blut-Hirn-Schranke sollte bewirken, dass EPO einfacher ins Hirngewebe eindringt. Insgesamt ist aber zu berücksichtigen, dass generell Einschränkungen tierexperimenteller Versuche gelten[86]. Das heißt, dass wie erwähnt eine Vorbehandlung stattfand, eine hohe Dosis verabreicht wurde, keine klinisch-funktionellen Unterschiede auftraten, das Infarktvolumen nur ein Surrogatparameter ist und nur ein kurzer Beobachtungszeitraum vorlag. Daher müsste eine klinische Testung mit Gabe von EPO bei Eintreffen in der Klinik bzw. nach abgeschlossener Akut-Diagnostik erfolgen, um einen klinischen Effekt zu beurteilen. Ein weiteres Anwendungsfeld könnte eine Gabe von EPO vor Eingriffen mit potenziellem Schlaganfallrisiko sein, wie beispielsweise vor Eingriffen mit einer Herz-Lungen-Maschine oder Carotisendarteriektomie.

In beiden Studien zur FEES bei Schlaganfallpatienten sowie neurologischen Intensivpatienten zeigte sich bei einem hohen Teil der Patienten das Vorliegen einer Dysphagie. Dennoch hatte ein Großteil der Patienten keine ihren Fähigkeiten angepasste Ernährung. Dies unterstreicht zum einen die Notwendigkeit von instrumenteller Diagnostik bei diesen Patienten und den kontinuierlichen Schulungsbedarf bezüglich Dysphagien bei Pflegenden und Therapeuten, inklusive dem ärztlichen Personal. Die Anpassung der Ernährung kann dabei entweder bei Reduktion der Einschränkungen zu einer besseren Ernährungssituation und Lebensqualität führen oder Komplikationen vermeiden. Wir beobachteten zumindest bei den Schlaganfallpatienten weniger Pneumonien. Bei den Intensivpatienten konnte dieser Effekt nicht beobachtet werden, da aufgrund des retrospektiven Studiendesigns auch Patienten mit beispielsweise präklinischer Aspiration ebenfalls berücksichtigt wurden. Zusätzlich konnten Patienten mit Anpassung der Ernährung früher aus der Klinik entlassen werden, was zum einen zu geringeren Behandlungskosten und zum anderen zu einem potenziell belegbarem „freien Bett“ in der Klinik führt. Die FEES stellt insgesamt eine sichere Untersuchungsmethode direkt am Patientenbett dar, die eine großes Potential bietet, Komplikationen zu vermeiden, wenn sie breit angewendet wird. Seit dem Arbeitsbeginn des Autors 2012 setzt sich die FEES zunehmend im klinischen Alltag in der Neurologie durch. Damals waren die FEES und die

Dysphagie noch als Exotikum angesehen. Seitdem jedoch kontinuierlich das Personal unserer Klinik geschult wurde und an den Untersuchungen als Beobachter teilnahm, wuchs zunehmend das Vertrauen in die Untersuchung. Mittlerweile wird sie routinemäßig angewandt. Auch wurden auf Dysphagien spezialisierte Logopäden angestellt, um die Versorgung der Patienten zu verbessern. Als eine der ersten Maßnahmen wurde ein standardisiertes Dysphagiemanagement etabliert. Die Logopäden führen die FEES überwiegend selbstständig in hoher Qualität durch. Eine Untersuchung aus dem Jahr 2017 zeigte, dass in Deutschland mittlerweile 70 Prozent der Stroke Units die FEES einsetzen[63]. Auch gibt es seit dem Jahr 2014 ein Curriculum für die FEES mit der Möglichkeit ein Zertifikat analog zu den Diagnostikzertifikaten der Deutschen Gesellschaft für Klinische Neurophysiologie oder der Deutschen Gesellschaft für Ultraschall in der Medizin zu erwerben[207].

In der PHAST-TRAC-Studie wurde die PES bei schwer betroffenen Schlaganfallpatienten mit Tracheotomie untersucht. Dabei war die PES einer Scheinstimulation deutlich überlegen[208]. Die PES wird damit sicherlich in der Zukunft eine sehr interessante Behandlungsmethode für Dysphagiepatienten darstellen. In der PHAST-TRAC-Studie profitierten vor allem jüngere Patienten, Patienten mit niedrigerem Schweregrad des Schlaganfalls und Patienten, die frühzeitig stimuliert wurden. Im Gegensatz zur PHAST-TRAC-Studie zeigten die Patienten der STEPS-Studie, bei der das gleiche Gerät zur Stimulation genutzt wurde, keinen Effekt der Therapie[77]. Daher muss weiter erforscht werden, welche Patienten von einer PES profitieren. Dies ist insbesondere vor dem Hintergrund zu sehen, dass die Durchführung der Therapie mit hohen Kosten verbunden ist. Insbesondere ist weitere Forschung bei nicht-tracheotomierten Patienten notwendig, da es wenig logisch erscheint, dass Patienten die geringer betroffen sind (da sie nicht einer Behandlung auf der Intensivstation bedürfen), nicht von einer solchen Therapie profitieren sollen.

Zusammenfassend zeigt sich, dass für unterschiedliche Szenarien in der Akut- und Postakutphase Therapieansätze bestehen, die weiter untersucht werden sollten. Aktuell verfolgen die Leitlinien einen „One-size-fits-all“-Ansatz, der so nicht zu halten zu sein scheint, sodass im klinischen Alltag sich Therapieentscheidungen häufig im Rahmen der Freiräume existierender klinischer Leitlinien bewegen. Insgesamt entstand in den letzten Jahren der Eindruck, dass ein einheitliches Vorgehen, wie in klinischen Studien oder den aktuellen Leitlinien festgehalten, immer weniger praktikabel ist, sondern dass zunehmend mehr individuelle Therapieentscheidungen getroffen werden sollten[34]. In der Akutphase wird sich wahrscheinlich die Selektion von Patienten für Thrombektomie und systemische Lyse immer weiter von den ursprünglichen Einschluss- und Zulassungsbedingungen entfernen und eine Entscheidung wird aufgrund des Vorhandenseins von rettbarem Gewebe getroffen werden. Diese Trends werden bei der Erstellung neuer Studien berücksichtigt werden müssen. Inwieweit dann ein Vorgehen noch operationalisiert werden kann, bleibt abzuwarten.

Insgesamt wäre es daher empfehlenswert, dass nicht *der* Schlaganfallpatient im Allgemeinen in Studien untersucht wird, sondern dass Studiendesigns unterschiedliche Situationen abdecken und so individualisiertere Gruppen von Patienten in Studien gebildet werden. Ein solcher Ansatz eröffnet den Behandlern die Möglichkeit, sich von einem starren Vorgehen zu entfernen und an die Patientensituation angepasst vorzugehen. Da die Therapie der Dysphagie entsprechend dem individuellen Pathomechanismus erfolgt, gelten hier analog die gleichen Bedingungen.

5. Eigene Originalarbeiten

5.1. Inter and intra-rater reliability of computer-assisted planimetry in experimental stroke research.[209]

Tobias Braun, Jan Pukropski, Mesut Yeniguen, Jasmin El-Shazly, Markus Schoenburg, Tibo Gerriets, Manfred Kaps, Marlene Tschernatsch, Martin Juenemann

Eine häufig angewandte Möglichkeit der Überprüfung des Erfolgs in der experimentellen Schlaganfallforschung ist die Bestimmung der Größe des Schlaganfalls. In der experimentellen Schlaganfallforschung ist die computergestützte Planimetrie zur Bestimmung der Größe einer ischämischen Läsion oder des Hemisphärenvolumens eine weit verbreitete Methode. Jedoch existieren nur wenige Daten darüber, wieviel Einarbeitung und Training notwendig sind, um eine ausreichende Reliabilität bei der Durchführung der Methode zu erreichen. Daher wurde verfolgt, wie sich die Übereinstimmung von zwei verblindeten Untersuchern, die neu in die Planimetrie eingearbeitet wurden, über 15 Monate veränderte. Zur Untersuchung der Inter-Rater-Reliabilität wurden das Hemisphären- und ischämische Läsionsvolumen anhand von T2- und diffusions-gewichteten MRT-Sequenzen von 227 männlichen Wistar Unilever Ratten, bei denen experimentell ein Verschluss der A. cerebri media erzeugt wurde, untersucht. Zur Untersuchung der Intra-Rater-Reliabilität bestimmte ein Untersucher zweimalig das Hemisphären- und das ischämische Läsionsvolumen in 87 T2-gewichteten MRT-Sequenzen im Abstand von 6 Wochen. Die Korrelation wurde mittels Krippendorff-Alpha berechnet und es wurden Bland-Altman-Plots angefertigt, um die Übereinstimmung darzustellen.

Das Inter-Rater-Agreement stieg innerhalb der ersten sieben Wochen an und blieb auf vergleichbar hohen Werten (Krippendorff-Alpha > 0,88). Das Krippendorff-Alpha des Intra-Rater-Agreements lag bei 0,84 für das Hemisphären- und bei 0,85 für das ischämische Läsionsvolumen. Die Bland-Altman-Plots zeigten ein gutes Agreement zwischen den Untersuchern. Ein Anhalt für systematische Verzerrung bestand nicht.

Alternativ zur Planimetrie können auch vereinfachte geometrische Modelle oder auch automatisierte Planimetriemethoden genutzt werden, die jedoch nicht adäquat auf das Hemisphärenvolumen angewendet werden können.

Die computergestütze Planimetrie stellt eine adäquate Methode zur Bestimmung des Hemisphären- und Läsionsvolumen bei Nagetieren dar, benötigt aber eine ausreichend lange Einarbeitungszeit von ca. zwei Monaten. Unsere Ergebnisse zeigten, dass auch ein erfahrener Untersucher Daten mit zum Teil erheblichen Varianzen erzeugen kann. Daher sollten Inter-

und Intra-Rater-abhängige Abweichungen bei der Planung und Durchführung von entsprechenden Studien berücksichtigt werden.

Journal of Neuroscience Methods 312 (2019) 12–15

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Short communication

Inter- and intra-rater reliability of computer-assisted planimetry in experimental stroke research

Tobias Braun[a,*], Jan Pukropski[a], Mesut Yeniguen[a], Jasmin El-Shazly[a], Markus Schoenburg[b], Tibo Gerriets[a,c], Manfred Kaps[a], Marlene Tschernatsch[a,c], Martin Juenemann[a]

[a] Department of Neurology, Heart & Brain Research Group, University Hospital Giessen and Marburg, Klinikstrasse 33, 35392, Giessen, Germany
[b] Department of Heart Surgery, Kerckhoff Heart and Thorax Center, Benekestrasse 2-8, 61231, Bad Nauheim, Germany
[c] Department of Neurology, Gesundheitszentrum Wetterau, Chaumontplatz 1, 61231, Bad Nauheim, Germany

ARTICLE INFO

Keywords:
Planimetry
Volumetry
Infarct size analysis
Experimental stroke
MRI
Reliability

ABSTRACT

Background: Computer-assisted planimetry is widely used in experimental stroke research to assess the size of the ischemic lesion or hemispheric volume.
New method: Only insufficient data exist on the training required to achieve sufficient reliability in planimetry. Therefore, planimetry was performed over 15 months by two blinded raters who were initially inexperienced in the method. For inter-rater reliability, the hemispheric and lesional volume of 227 male Wistar Unilever rats subjected to middle cerebral artery occlusion were determined in diffusion- and T2-weighted sequences. For the intra-rater agreement, one investigator assessed the hemispheric and lesional volume in 87 T2-weighted sequences twice within a six-week interval. The correlation was calculated using Krippendorff's alpha and Bland-Altman plots illustrated the agreement.
Results: Inter-rater agreement increased during the first seven weeks and remained at high values (Krippendorff's alpha > 0.88). For intra-rater agreement, Krippendorff's alpha was 0.84 for hemispheric and 0.85 for lesional volume. The Bland-Altman plot indicated solid agreement between raters in the absence of systematic errors.
Comparison with existing methods: Simplified geometrical models or automated methods for planimetry can be used to determine lesional volume, but both approaches are inappropriate to assess hemispheric volume.
Conclusion: Computer-assisted planimetry can be an appropriate method to determine hemispheric or ischemic lesion volume in rodents but requires a sufficiently long learning period of approximately two months. Even an experienced investigator can generate data with serious variation. Inter- and intra-rater-dependent bias should be considered during the design and performance of respective studies.

1. Introduction

Computer-assisted planimetry is a widely established tool that is used to determine hemispheric volume (HV) or ischemic lesion volume in experimental stroke research. For the assessment of clinical deficits, scaled sensomotoric tests for different deficits, or rather global tests, such as the rotarod test, are often used. However, the clinical evaluation of animals often proves impractical and due to the fact that a blinded, trained tester is needed; those tests are often prone to subjectivity and variation. For example, a test in which a reflex-paw placement is elicited by touching the ipsilateral vibrissae on a hard surface may be complicated in mice due to the animal's resistance to being positioned in such a way, resulting in variability and unreliable assessments due to animal mobility, temperament or motivation (Kahle and Bix, 2012). Another problem is the fact that some deficits – neglect, for example - might be hard to detect or might develop over time (such as post-stroke dysphagia) (Sugiyama et al., 2014). In contrast, the size of an ischemic lesion seems to be less affected by confounding factors, and, therefore, ischemic lesion volume is often used as a robust primary endpoint for the assessment of therapeutic efficacy (Kahle and Bix, 2012).

Computer-assisted planimetry can be applied in computed tomography, magnetic resonance (MR) or histological staining techniques, such as tetrazolium chloride staining (Okuno et al., 2001; Sims et al., 2009). Based on the Cavalieri principle, a set of two-dimensional, parallel slices of an object with known thickness is needed. To calculate the volume of the object, the area of each slice is determined manually.

* Corresponding author at: Department of Neurology, University Hospital Giessen and Marburg, Klinikstrasse 33, 35392, Giessen, Germany.
E-mail address: Tobias.Braun@neuro.med.uni-giessen.de (T. Braun).

https://doi.org/10.1016/j.jneumeth.2018.11.012
Received 18 August 2018; Received in revised form 17 November 2018; Accepted 17 November 2018
Available online 19 November 2018
0165-0270/

Then, the areas are added and multiplied by the thickness of the slices (Roberts et al., 2000). Geometric approaches – e.g., ellipsoid or spherical models – can be used to estimate infarct size as well, but these formulae tend to over- or underestimate the true volume (Sims et al., 2009).

Our investigation was conducted to assess the inter- and intra-rater reliability of planimetry in stroke-related experimental MR imaging.

2. Methods

To calculate inter- and intra-rater reliability, we prospectively assessed imaging data in experimental stroke research in our own research group over 15 months. All procedures were conducted in accordance with our institutional guidelines and the German animal protection legislation and were approved by the regional ethics committee (Regierungspraesidium Darmstadt: V54-19c 20/15-B2/170; V54-19c 20/15-B2/144).

For inter-rater reliability, HV and lesional volume (LV) of a total of 227 male Wistar Unilever rats (HsdCpb:WU; Harlan Winkelmann, Germany) were determined in diffusion-weighted (DWI) and T2-weighted sequences. In short, imaging was performed using a seven Tesla MRI device (Bruker PharmaScan 7.0 T, 16 cm). Overall, 214 DWI sequences and 278 T2-weighted sequences were analysed in this manner.

All animals were subjected to middle cerebral artery occlusion (MCAO) using the suture model (Koizumi 1986): Prior to surgery, each rat was administered 100 mg/kg metamizol (Novalgin®, Sanofi, Germany) orally. Anaesthesia was administered with 5% isoflurane delivered in air at 3.0 L/min and maintained during surgery via a facial mask with 2–3% isoflurane delivered in air at 0.5 L/min. Body core temperature was recorded with a feedback-controlled heating pad and kept at 37.0 °C (± 0.25 °C) during surgery and imaging procedures. After local anaesthesia (2% lidocain [Xylocain®, AstraZeneca, Germany]), MCAO was performed. In brief, the right common carotid artery was exposed, and a silicone-coated nylon suture (4-0) was inserted. Then, the occluder was advanced proximally until its tip reached the anterior cerebral artery beyond the carotid bifurcation, thus blocking the blood flow to the right middle cerebral artery. Metamizol was administered orally again 6 h after the first application and applied to the tap water for the remaining survival time of 24 h.

Computer-assisted planimetry of the ischemic lesions and total HV was performed by two blinded investigators who had just learned this technique using the image analysis software Image J 1.25 s (National Institutes of Health, USA) (Schneider et al., 2012). First, contrast and brightness were adjusted in such a way that the borders of anatomical structures and the hyperintense ischemic lesions were clearly distinguishable. Afterwards, the edges of the hemispheres and the hyperintense ischemic lesions were traced manually on each slice using a handheld mouse and neuroanatomic landmarks (Fig. 1, a). The areas were then added and multiplied by the slice thickness to calculate corresponding volumes. Planimetry was demonstrated to the investigators once; they then performed planimetry three times under the supervision of an experienced user. Furthermore an example selection of MRI images displaying ischemic lesions, intracerebral haemorrhage, subarachnoidal haemorrhage and different artefacts (e.g., partial volume effects, ghosting or aliasing) was demonstrated to the raters. If these artefacts were judged by both investigators as severe and potentially impeding identification of lesion edges (e.g., missing parts of a hemisphere or lesion due to the artefact), corresponding images were excluded from the study. The training session lasted a total of 90 min. Because both investigators were new to planimetry, learning curves could be generated over the course of 15 months. For intra-rater agreement, one blinded investigator, with at least 18 months of experience in planimetry, examined the HV and LV in 87 T2-weighted sequences twice over a six-week interval.

Statistics were calculated using SPSS Version 19.0 for Windows (Armonk, NY: IBM Corp.). Inter- and intra-rater correlation was assessed with Krippendorff's alpha using an SPSS macro (http://www.afhayes.com/public/kalpha.sps). Krippendorff's alpha is a correlation coefficient ranging between -1 and +1. A value of +1 implies total agreement, whereas 0 implies no agreement. In nominal- and ordinal-scaled data, a Krippendorff's alpha of -1 can be achieved when the investigators form a contrary opinion, implying high reliability (Hayes and Krippendorff, 2007).

The Bland-Altman plot, a graphic tool based on a coordinate system, was used for each sequence and in HV and LV to visualize and estimate the true value, systematic errors and agreement, respectively (Bland and Altman, 1986). The average of the paired values (on the x-axis) was plotted against their difference (on the y-axis). The average was used, as it fluctuates around the true value, providing its best estimation. The mean of all measurements was plotted on the coordinate system using a horizontal line. Additionally, the limits of agreement (limits of agreement = average ± 2 * standard deviation) were added. The distribution of data points indicates systematic errors (Bland and Altman, 1986; Grouven et al., 2007).

3. Results

During the first seven weeks, planimetry was performed in 142 MRI sequences twice by each rater (89 T2-weighted & 53 DWI). The remaining analyses – i.e., 350 sequences for each rater – were evenly spread over the course of 60 weeks. All images analysed for this study were free of relevant artefacts. Both raters completed the same number of analyses at the same frequency and delayed those analyses only by a few hours. Krippendorff's alpha for inter-rater agreement over the course of 15 months for HV and LV in T2-weighted and DWI sequences reached their highest values, meaning in the highest agreement, after seven weeks (Fig. 1b).

Table 1 lists the means and limits of agreement used to calculate the Bland-Altman plots. Fig. 1c shows an exemplary Bland-Altman plot for the HV measured in T2-weighted sequences. Because Krippendorff's alpha reached its highest value after a training period of seven weeks, the values from the first seven weeks were removed, resulting in 82 T2-weighted and 108 DWI sequences; the plots were recalculated from the remaining data (Fig. 1d).

Table 1 depicts the values used in the Bland-Altman plots. The means of all differences were calculated. The limits of agreement were then calculated using the formula "mean of differences + 1.96 x standard deviation" for the upper limit and the formula "mean of differences – 1.96 x standard deviation" for the lower limit. The width of the limits of agreement can be used to estimate the extent of agreement.

4. Discussion

The use of computerised procedures in experimental stroke research suggests a certain degree of objectivity, but nevertheless, during the process of data acquisition, examiner dependence has to be assumed. Herein, we investigated if rater-dependent variations exist in computer-assisted planimetry.

This analysis showed that inter-rater agreement reached a high level of agreement after approximately two months or 284 examined MRI sequences and remained stable over the course of 15 months of observation (Fig. 1b, Krippendorff's alpha > 0.88). Because both investigators used the same technique and measured the same data, a strong correlation was expected (Grouven et al., 2007). However, a strong correlation cannot necessarily be equated with strong agreement. We therefore used the gold standard when assessing agreement: the Bland-Altman plot (Bland and Altman, 1986).

The Bland-Altman plots showed smaller limits of agreement when data from the learning period of seven weeks were excluded (Table 1, Fig. 1c + d). Any variation observed during this period revealed no relationship with the volume measured. All Bland-Altman plots showed

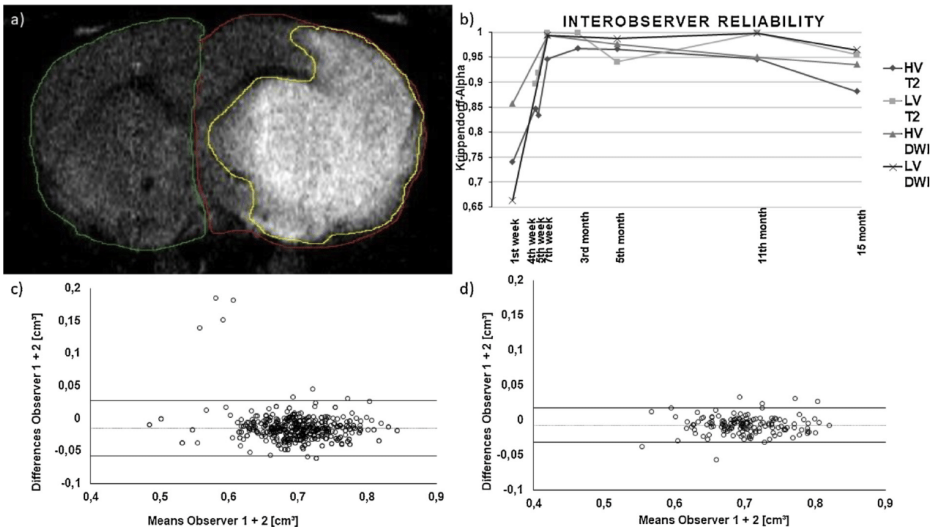

Fig. 1. a) Computer-aided planimetry, T2-weighted MRI image. The yellow line traces the ischemic lesion, the red line traces the ipsilateral hemisphere and the green line traces the contralateral hemisphere b) Inter-rater agreement is expressed as Krippendorff's alpha over the course of 15 months for HV and LV in T2-weighted and DWI sequences, respectively. Krippendorff's alpha reached the highest value – i.e., the strongest agreement – after seven weeks. Finally, c) and d) show two exemplary Bland-Altman plots. c) The complete data set for HV in T2-weighted images. d) Data from the first seven-week period were removed, visualising a considerable narrowing of the limits of agreement. Further Bland-Altman plots are not presented, because they showed similar results.

Table 1
Calculated Values for the Bland-Altman Plots.

Intra-rater	Mean of differences [cm³]	Limit of agreement [cm³]	
		Upper	Lower
HV T2-weighted sequences	−0.006	0.094	−0.107
LV T2-weighted sequences	0.006	0.110	−0.096
HV T2-weighted sequences incl. the first 7-week period	−0.015	0.028	−0.058
HV T2-weighted sequences w/o the first 7-week period	−0.006	0.017	−0.031
HV Diffusion-weighted sequences incl. the first 7-week period	−0.006	0.051	−0.064
HV Diffusion-weighted sequences w/o the first 7-week period	−0.004	0.038	−0.029
LV T2-weighted sequences incl. the first 7-week period	−0.001	0.072	−0.074
LV T2-weighted sequences w/o the first 7-week period	0	0.014	−0.014
LV Diffusion-weighted sequences incl. the first 7-week period	−0.001	0.067	−0.063
LV Diffusion-weighted sequences w/o the first 7-week period	−0.002	0.015	−0.021

similar results for HV or LV in T2-weighted and DWI sequences. In all plots, the scatter of points did not suggest a systematic error such as large differences for small lesion size (cloud-shaped scattering in upper left corner) or large lesion size (cloud-shaped scattering in upper right corner).

Due to the observation that intra-rater agreement was less than inter-rater agreement, we hypothesize that even an experienced investigator can generate data with serious variations that must be considered during the study design and interpretation of planimetric data. An alternative interpretation might indicate an investigator's poor planimetry skills, although he had approximately 18 months of experience using planimetry.

Limited data exist on the inter- and intra-rater reliability of planimetry in experimental stroke research. Nagel and co-workers demonstrated good intra-rater reliability when using planimetry with MRI sequences. The reliability, when assessing the LV, was dependent on the time lag between MCAO and MRI, with a higher variability after 5 h from MCAO compared to 3, 8 or 12 h from MCAO. The variability of HV was lower and not time-dependent. However, the authors did not report on the experience of the rater (Nagel et al., 2004). Friedländer and co-workers reported a lower reliability of planimetry compared to an ImageJ macro when using triphenyltetrazolium chloride staining. In this investigation, the results of 15 students "who were all familiar with […] ImageJ-based free-hand planimetry" were compared to the aforementioned macro. They did not indicate their time of training or expertise (Friedländer et al., 2017). Bråtane and co-workers reported a higher correlation when ischemic lesions on MRI images were determined by an experienced investigator rather than by an unexperienced investigator. Furthermore, the results showed better agreement when apparent diffusion coefficient (ADC) images were used instead of DWI images. The limits of agreement ranged from -42.4 to 62.1 mm³ (ADC) and from -71.1 to 125.5 mm³, which are larger than our values after the training period (Bråtane et al., 2009). This further supports the value of sufficient training.

Our data suggest that computer-assisted planimetry can be an appropriate method with which to determine HV or ischemic lesion volume in rodents, but it requires a sufficiently long learning period. A training period of approximately two months, including the analysis of

approximately 280 images, should be included in the experimental design. However, even after this learning period, the method remains prone to rater-dependent, systematic errors. In our experience, this is mostly due to different contrast parameters, especially along the border of the ischemic lesion and healthy brain tissue, and small variances when tracing the edges of an object using a handheld mouse, leading to deviations as a result of error propagation. When designing an experimental stroke trial, sufficient time should be allowed in advance for appropriate training of the rater, with images from past studies. A thoroughly conducted training of the raters should lead to smaller deviations and thus might facilitate smaller sample sizes. However, our study was not powered to address this issue.

Evaluating the validity of planimetry in experimental stroke research is hard to realise because there is no gold standard by which to quantify the exact volume of the hemisphere or ischemic regions, respectively. The HV can be quantified by its water displacement using Archimedes' principle. However, a transfer of this method to the irregularly and inhomogeneously shaped ischemic lesion is difficult. Because a means by which to test the validity of this method is hard to realise, we did not pursue this matter.

A different approach for volumetry is Euclidean geometry. Here, LV is approximately calculated using simple geometric models (sphere, ellipsoid, cylinder). Those models tend to over- or underestimate the true volume of the lesion. The spherical model seems to achieve the best results compared to planimetry (Sims et al., 2009). However, as mentioned before, ischemic lesions are usually shaped irregularly, so these models seem to oversimplify. Although it seems acceptable to use a spherical model for correspondingly shaped ischemic lesions, the shape of the ischemic lesion is usually – in our opinion – far too different from geometric models. In human stroke, the irregular form was demonstrated by Asdaghi et al. (Asdaghi et al., 2014).

Moreover, different automated methods for volumetry exist: LV can be measured using ADC thresholding. By defining an ischemic threshold of the ADC, an automated program can define the ischemic volume using these values on the MRI sequence (Meng et al., 2004). Alternatively, autotracing techniques have been evaluated that use grey values in MRI-images (Nagel et al., 2004). Both methods are fast, automated, and user-independent. However, they cannot be used when trying to assess the HV.

In summary, planimetry is a reliable approach for volumetry that requires sufficient training. When assessing LV, automated approaches seem to be a user-independent option by which to assess HV; there does not seem to be an alternative for planimetry. As such, sufficient skills and training in planimetry are needed in experimental stroke research. Minimisation of inter-rater-dependent bias in investigation with multiple arms could be achieved by using one single rater for all data or, if multiple raters are required, by an equal allocation of the different study groups. However, researchers must remember that even an experienced rater will, to some extent, generate data with serious variation. Computer-assisted planimetry is a fast method that could be even faster or even more accurate when using a stylus-based graphical tablet or a touch screen-based system. Further investigations may address this approach.

Funding

The study received no funding.

Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

Research involving animals

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures were conducted in accordance with our institutional guidelines and the German animal protection legislation and were approved by the regional ethics committee (Regierungspraesidium Darmstadt: V54-19c 20/15-B2/170; V54-19c 20/15-B2/144).

References

Asdaghi, N., Pearce, L.A., Nakajima, M., Field, T.S., Bazan, C., Cermeno, F., et al., 2014. Clinical correlates of infarct shape and volume in lacunar strokes: the secondary prevention of small subcortical strokes trial. Stroke 45 (10), 2952–2958.
Bland, J.M., Altman, D.G., 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet (London, England) 1 (8476), 307–310.
Bråtane, B.T., Bastan, B., Fisher, M., Bouley, J., Henninger, N., 2009. Ischemic lesion volume determination on diffusion weighted images vs. apparent diffusion coefficient maps. Brain Res. 1279, 182–188.
Friedländer, F., Bohmann, F., Brunkhorst, M., Chae, J.-H., Devraj, K., Köhler, Y., et al., 2017. Reliability of infarct volumetry: its relevance and the improvement by a software-assisted approach. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 37 (8), 3015–3026.
Grouven, U., Bender, R., Ziegler, A., Lange, S., 2007. Vergleich von messmethoden. Dtsch. Med. Wochenschr. (1946) (132 Suppl 1), e69–e73.
Hayes, A.F., Krippendorff, K., 2007. Answering the call for a standard reliability measure for coding data. Commun. Methods Meas. 1 (1), 77–89.
Kahle, M.P., Bix, G.J., 2012. Successfully climbing the "STAIRs": surmounting failed translation of experimental ischemic stroke treatments. Stroke Res. Treat. 2012, 374098.
Meng, X., Fisher, M., Shen, Q., Sotak, C.H., Duong, T.Q., 2004. Characterizing the diffusion/perfusion mismatch in experimental focal cerebral ischemia. Ann. Neurol. 55 (2), 207–212.
Nagel, S., Wagner, S., Koziol, J., Kluge, B., Heiland, S., 2004. Volumetric evaluation of the ischemic lesion size with serial MRI in a transient MCAO model of the rat: comparison of DWI and T1WI. Brain research. Brain Res. Protoc. 12 (3), 172–179.
Okuno, S., Nakase, H., Sakaki, T., 2001. Comparative study of 2,3,5-triphenyltetrazolium chloride (TTC) and hematoxylin-eosin staining for quantification of early brain ischemic injury in cats. Neurol. Res. 23 (6), 657–661.
Roberts, N., Puddephat, M.J., McNulty, V., 2000. The benefit of stereology for quantitative radiology. Br. J. Radiol. 73 (871), 679–697.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9 (7), 671–675.
Sims, J.R., Gharai, L.R., Schaefer, P.W., Vangel, M., Rosenthal, E.S., Lev, M.H., et al., 2009. ABC/2 for rapid clinical estimate of infarct, perfusion, and mismatch volumes. Neurology 72 (24), 2104–2110.
Sugiyama, N., Nishiyama, E., Nishikawa, Y., Sasamura, T., Nakade, S., Okawa, K., et al., 2014. A novel animal model of dysphagia following stroke. Dysphagia 29 (1), 61–67.

5.2. Neuroprotective mechanisms of erythropoietin in a rat stroke model[206]

Martin Juenemann*, **Tobias Braun***, Nadine Schleicher, Mesut Yeniguen, Patrick Schramm, Tibo Gerriets, Nouha Ritschel, Georg Bachmann, Martin Obert, Markus Schoenburg, Manfred Kaps, Marlene Tschernatsch

*contributed equally

Die Erfolge zahlreicher präklinischer tierexperimenteller Studien zur Neuroprotektion nach einem Schlaganfall ließen sich in der Vergangenheit nicht in klinischen Studien beim Menschen nachweisen. Daher ist auch das vasogene Ödem, das für bis zu 50 Prozent des endgültigen ischämischen Läsionsvolumens verantwortlich ist, zunehmend in der Fokus der präklinischen Forschung gerückt. Durch Verminderung des vasogenen Ödems soll das definitve Infarktvolumen verringert werden. Dieser Ansatz wurde als indirekte Neuroprotektion bezeichnet.

Das Ziel dieser Studie war es, zu untersuchen, ob rekombinantes humanes Erythropoetin (rhEPO) indirekte neuroprotektive Eigenschaften in einem Hirninfarktmodell mit transientem Verschluss der A. cerebri media (MCAO) besitzt.

Hierfür wurden 110 männliche Wistar-Ratten in vier Gruppen randomisiert, die entweder 5.000 IU/kg rhEPO oder die gleiche Menge an Kochsalzlösung 15 Minuten vor MCAO und entweder eine bilaterale Kraniektomie oder eine Schein-Kraniektomie erhielten. Die bilaterale Hemikraniektomie sollte den raumfordernden Effekt des postischämischen vasogenen Ödems eliminieren. Die durchgeführte Diagnostik umfasste eine neurologische Untersuchung, die Messung der Infarktgröße und des zerebralen Ödems mittels MRT-Bildgebung, die Messung des hemisphärischen und lokalen cerebralen Blutflusses (CBF) mittels flat-panel-Volumen-Computertomographie sowie die Bestimmung des nass/trocken-Gewichts.

Ohne Kraniektomie zeigte sich, dass eine Vorbehandlung mit EPO zu einer signifikanten Reduktion des Infarktvolumens (34,83+/-9,84% vs. 25,28+/-7,03% p=0,022) und der Mittellinienverlagerung (0,114+/-0,023cm vs. 0,083+/-0,027cm p=0,013) führte. Wir beobachteten eine signifikante Erhöhung im zerebralen Blutfluss in kortikalen Regionen des Infarktgebietes (72,29+/-24,00% vs. 105,53+/-33,10% p=0,043), jedoch nicht in den gesamten Hemisphären. Parameter, die unabhängig von der Infarktgröße waren, führten nicht zu einer statistisch signifikanten Abnahme des Hirnödems bei Tieren, die mit rhEPO behandelt wurden.

Zusammenfassend beobachteten wir bei der Ratte eine signifikante Reduktion des ischämischen Infarktvolumens und eine Erhöhung des lokalen CBF in den Penumbragebieten

der Ischämie 24 Stunden nach Infarktinduktion nach einer einmaligen Vorbehandlung mit 5000IU/kg rhEPO. Unsere Daten legen eine indirekte Neuroprotektion vor dem Ödem mit einer daraus resultierenden Reduktion des intrazerebralen Drucks und Erhöhung des Blutflusses vermittelt durch EPO nahe.

Research Article

Martin Juenemann#, Tobias Braun#, Nadine Schleicher, Mesut Yeniguen, Patrick Schramm, Tibo Gerriets, Nouha Ritschel, Georg Bachmann, Martin Obert, Markus Schoenburg, Manfred Kaps, Marlene Tschernatsch*

Neuroprotective mechanisms of erythropoietin in a rat stroke model

https://doi.org/10.1515/tnsci-2020-0008
received May 06, 2019; accepted January 02, 2020

Abstract

Objective – This study was designed to investigate the indirect neuroprotective properties of recombinant human erythropoietin (rhEPO) pretreatment in a rat model of transient middle cerebral artery occlusion (MCAO).

Methods – One hundred and ten male Wistar rats were randomly assigned to four groups receiving either 5,000 IU/kg rhEPO intravenously or saline 15 minutes prior to MCAO and bilateral craniectomy or sham craniectomy. Bilateral craniectomy aimed at elimination of the space-consuming effect of postischemic edema. Diagnostic workup included neurological examination, assessment of infarct size and cerebral edema by magnetic resonance imaging, wet–dry technique, and quantification of hemispheric and local cerebral blood flow (CBF) by flat-panel volumetric computed tomography.

Results – In the absence of craniectomy, EPO pretreatment led to a significant reduction in infarct volume (34.83 ± 9.84% vs. 25.28 ± 7.03%; $p = 0.022$) and midline shift (0.114 ± 0.023 cm vs. 0.083 ± 0.027 cm; $p = 0.013$). We observed a significant increase in regional CBF in cortical areas of the ischemic infarct (72.29 ± 24.00% vs. 105.53 ± 33.10%; $p = 0.043$) but not the whole hemispheres. Infarct size-independent parameters could not demonstrate a statistically significant reduction in cerebral edema with EPO treatment.

Conclusions – Single-dose pretreatment with rhEPO 5,000 IU/kg significantly reduces ischemic lesion volume and increases local CBF in penumbral areas of ischemia 24 h after transient MCAO in rats. Data suggest indirect neuroprotection from edema and the resultant pressure-reducing and blood flow-increasing effects mediated by EPO.

Keywords: neuroprotection, rat, recombinant human erythropoietin, transient focal cerebral ischemia, vascular disorders, craniectomy

1 Introduction

To date, many studies have been conducted on the identification, development, and evaluation of pharmacological neuroprotectants. In this context, experimental

# Both authors contributed equally to this work.

*** Corresponding author: Marlene Tschernatsch,** Department of Neurology, Justus-Liebig-University Giessen, Klinikstrasse 33, 35392, Giessen, Germany; Heart & Brain Research Group, Justus-Liebig-University Giessen and Kerckhoff Clinic, Benekestrasse 2-8, 61231, Bad Nauheim, Germany; Department of Neurology, Gesundheitszentrum Wetterau, Chaumontplatz 1, 61231, Bad Nauheim, Germany, e-mail: marlene.tschernatsch@neuro.med.uni-giessen.de
Martin Juenemann, Tobias Braun, Mesut Yeniguen, Patrick Schramm: Department of Neurology, Justus-Liebig-University Giessen, Klinikstrasse 33, 35392, Giessen, Germany; Heart & Brain Research Group, Justus-Liebig-University Giessen and Kerckhoff Clinic, Benekestrasse 2-8, 61231, Bad Nauheim, Germany
Nadine Schleicher: Heart & Brain Research Group, Justus-Liebig-University Giessen and Kerckhoff Clinic, Benekestrasse 2-8, 61231, Bad Nauheim, Germany
Tibo Gerriets: Department of Neurology, Justus-Liebig-University Giessen, Klinikstrasse 33, 35392, Giessen, Germany; Heart & Brain Research Group, Justus-Liebig-University Giessen and Kerckhoff Clinic, Benekestrasse 2-8, 61231, Bad Nauheim, Germany; Department of Neurology, Gesundheitszentrum Wetterau, Chaumontplatz 1, 61231, Bad Nauheim, Germany
Nouha Ritschel: Max-Planck-Institute for Heart and Lung Research, Ludwigstraße 43, 61231, Bad Nauheim, Germany
Georg Bachmann: Department of Radiology, Kerckhoff Clinic, 61231, Bad Nauheim, Germany
Martin Obert: Department of Radiology, Justus-Liebig-University Giessen, Klinikstrasse 33, 35392, Giessen, Germany
Markus Schoenburg: Department of Cardiac Surgery, Kerckhoff Clinic, 61231, Bad Nauheim, Germany
Manfred Kaps: Department of Neurology, Justus-Liebig-University Giessen, Klinikstrasse 33, 35392, Giessen, Germany

research has demonstrated that systemically administered recombinant human erythropoietin (rhEPO) partially crosses the blood–brain barrier (BBB) with a latency and is able to reduce neuronal damage in animal models of cerebrovascular, neuroinflammatory, and neurodegenerative diseases as well as traumatic central nervous system (CNS) injury [1–4]. Depending on the study design, preclinical studies on EPO in stroke models indicate an improvement in infarct size by up to 32% and neurobehavioral outcomes by almost 40% [5,6].

Erythropoietin production in the CNS seems to be triggered by hypoxia. Astrocytes, as well as oligodendrocytes, endothelial cells, neurons, and microglia, endogenously produce EPO. In principle, several types of EpoR receptors exist, including the homodimeric receptor $(EpoR)_2$, a soluble as well as a heterodimeric receptor comprising a functional interaction of EpoR with the common β receptor (βcR, also known as CD131). The homodimeric EpoR has been detected on neural progenitor cells (NPCs), neurons, astrocytes, endothelial cells, and microglia [7–9]. Upregulation of EPO and EpoR in infarct and peri-infarct regions has been demonstrated in the course of focal cerebral ischemia/hypoxia [10]. The interaction of EPO and its receptor induces the phosphorylation of Janus kinase 2, which leads to the activation of phosphoinositide 3-kinase–serine–threonine kinase Akt and/or the signal transducer and activator of transcription 5 and/or the nuclear factor-κB pathway [11]. In this system, the EPO may exert neuroprotective effects via antiapoptotic mechanisms, stimulation of NPC proliferation and differentiation, neurogenesis, angiogenesis, and modification of inflammatory response and also induces erythropoiesis [7,8,12]. The heterodimeric EpoR/βcR receptor has been detected in various EPO-responsive tissues, including the cells of the CNS, such as microglia, and of the heart and kidney. It has been shown that this coexpression mediates the tissue-protective properties of EPO rather than erythropoietic effects [13].

Neuroprotection consists of prevention and opposition of pathological neuronal loss in diseases of the CNS [14]. Within cerebral ischemia, this loss can only partly be attributed to vessel occlusion. Moreover, perfusion deficits and the adjacent functional decline following cerebral vessel occlusion are consequences of the space-occupying effect of postischemic cerebral edema. Experimental data suggest that tissue swelling due to vasogenic edema during the hyperacute phase (<6 h) of stroke has considerable influence on temporospatial progression of the ischemic area by compromising microcirculation within critically perfused tissue at risk [15–18]. Therapeutic measures aiming at reducing cerebral edema and its space-occupying effect in the early stages of stroke may therefore induce an indirect "secondary" neuroprotection [16].

Most experimental studies focused on EPO treatment within the first hours following vessel occlusion [7], simulating the unpredictable situation clinicians face in the emergency department or in the stroke unit after sudden onset of a neurological deficit. However, with the advent of interventions in the cardio- and cerebrovascular systems – such as carotid endarterectomy and stenting, coronary artery bypass grafting, percutaneous coronary and cerebrovascular thrombectomy, angioplasty or coiling, and clipping of cerebral aneurysms – that carry an increased risk of stroke or require transient cerebral artery occlusion [19–26], anticipatory neuroprotection preceding a risk-related procedure demands greater attention [27]. In this context, experiments on a rodent model for transient middle cerebral artery occlusion (MCAO) suggest that beneficial effects of EPO treatment before ischemia onset can have a definite (if indirect) impact on the extent of ischemic edema and preservation of BBB function [27].

This study was designed to investigate secondary neuroprotective properties of rhEPO treatment preceding transient MCAO in a rodent stroke model. Dosage (5,000 IU/kg) and intravenous application were chosen according to the findings from the corresponding in vivo studies, considering the significantly low BBB permeability of this compound [1,7,28]. The multimodal approach included magnetic resonance imaging (MRI), flat-panel volumetric computed tomography (fpVCT), and quantification of brain water content (BWC) by the wet–dry technique. Elimination of the space-occupying effect of cerebral edema was achieved by bilateral craniectomy [29].

2 Methods

2.1 Animal preparation and surgical procedures

Male Wistar Unilever rats (HsdCpb:WU; Harlan Winkelmann, Germany) with a mean body weight of 310 g (±19.47 g) were used. Prior to surgery, each rat was administered 100 mg/kg metamizole (Novalgin®; Sanofi, Germany) orally. Anesthesia was established with 5% isoflurane delivered in air at 3.0 L/min and maintained

during surgery via a facial mask with 2–3% isoflurane delivered in air at 0.5 L/min. The core body temperature was recorded with a feedback-controlled heating pad and kept at 37.0°C (±0.25°C) during surgery and imaging procedures.

In addition to considerable neurological deficits, rodents often exhibit pronounced cardiorespiratory instability after occlusion of the middle cerebral artery (MCA). Since this study was not intended to evaluate the craniectomy itself, but rather the effect of EPO under various pressure conditions, the craniectomy was performed before MCAO to avoid provoking an increased dropout rate through additional anesthesia and intervention with an already potentially unstable animal. Bilateral or sham craniectomies were performed after local anesthesia (2% lidocaine; Xylocaine®, AstraZeneca, Germany), as described previously [29]. The whole os parietale and the caudal parts of the os frontale were removed using a liquid-cooled trephine, while the dura mater was left intact.

Afterward, the animals were randomized to treatment with EPO or placebo and MCAO was performed in each rat as discussed previously [15]. In brief, the right common carotid artery was exposed and a silicone-coated nylon suture (4-0) was inserted. Then the occluder was advanced proximally until its tip reached the anterior cerebral artery (mean suture depth: 20 ± 2 mm) beyond the carotid bifurcation, thus blocking the blood flow to the right MCA. Reperfusion was established after 90 minutes by removing the suture. Metamizole was administered orally again 6 h after the first application and added to the tap water.

Ethical approval: The research related to animal use has been complied with all the institutional guidelines and the current German animal protection law. The experiments were approved by the regional committee for the care and use of animals (Regierungspraesidium Darmstadt; Az.B2/170).

2.2 Experimental setup

One hundred and ten rats were randomly assigned to four groups: (i) placebo + craniectomy, (ii) EPO + craniectomy, (iii) placebo–craniectomy, and (iv) EPO–craniectomy.

Craniectomy was performed in 56 animals (+craniectomy); the bone skull of 54 rats was thinned but not completely removed (–craniectomy). Thereafter, all 110 rats were randomly subjected to the treatment groups (EPO vs. placebo): 15 minutes prior to MCAO, each animal was administered 5,000 IU/kg EPO (NeoRecormon®; Roche, Germany) in 2 ml isotonic saline (EPO) or only 2 ml isotonic saline (placebo) via coccygeal venous catheter. Afterward, MCAO was performed by a surgeon blinded to the group assignment. Functional testing took place at baseline and 24 h after MCAO. Then ten rats of each group were subjected to MRI to detect ischemic lesion volume, vascular edema, and midline shift (MLS) and to post-mortem quantification of BWC by the wet–dry technique. The remaining animals of each group underwent quantification of cerebral blood flow (CBF) via fpVCT. Functional assessment, radiological imaging, and evaluation, as well as wet–dry analysis, were performed by experienced investigators blinded to the group assignment.

2.3 Functional testing

Motor functions were assessed using the Rotarod test at baseline and 24 h after MCAO. The wheel was continuously accelerated from 0 to 30 rpm within 1 minute. The maximum speed tolerated by the rats was documented and the difference was calculated as Rotarod performance before and after MCAO [30].

2.4 MRI

After functional testing, the MRI scanning was performed under anesthesia with a tomography (Bruker PharmaScan 7.0 T, 16 cm), which operates at 300.51 MHz (1H-imaging) and is equipped with a 300 mT/m self-shielding gradient system. The animal's respiratory rate was monitored noninvasively and maintained between 60 and 80/min by regulation of the isoflurane concentration.

The linear polarized volume resonator (diameter 60 mm) was tuned and matched manually, and localized images were acquired using a spin-echo sequence. Rapid acquisition with relaxation enhancement sequences (20 contiguous slices of 1 mm thickness, repetition time [TR] = 2500 ms, and echo time [TE] = 41.8 ms) were used to verify symmetric positioning and were repeated after correction of the possible necessary slice angulation [18].

2.5 T2-imaging

To map the vascular edema (T2-relaxation time [T2RT]) [15] and the lesion and hemispheric volumes, we used a Carr–Purcell–Meiboom–Gill spin echo imaging sequence,

acquiring eight contiguous coronal slices (slice thickness = 2 mm, gap = 0 mm, field-of-view (FOV) = 37 × 37 mm, matrix size = 512 × 256, TR = 3833.5 ms, TE [12 echos, ΔTE = 18 ms] = 18–216 ms, number of excitations (NEX) = 1, and acquisition time (AT) = 12 min 7 s).

2.6 T2*-imaging

To exclude animals with possible hemorrhages, 16 contiguous coronal slices were acquired using an SNAP-T2*-imaging sequence (slice thickness = 1 mm, gap = 0 mm, FOV = 37 × 37 mm, matrix size 256 × 256, TR = 43.4 ms, TE = 7.0 ms, and AT = 12 min 7 s).

2.7 MRI data evaluation

2.7.1 Ischemic lesion volume

The mean ischemic lesion volume was determined by performing computer-aided planimetric assessment of the lesion volume (LV) and the hemispheric volumes of the T2-weighted images (ipsilateral: HV_i; contralateral: HV_c) (ImageJ v1.46; National Institutes of Health, Bethesda, USA). The edema-corrected lesion volume ($\%HLV_{ec}$) was calculated by the following equation [18]:

$$\%HLV_{ec} = ((HV_c - HV_i + LV)/HV_c) \times 100 \quad (1)$$

2.7.2 MLS quantification

The MLS quantification was performed using high-resolution T2-weighted images. The position of the third ventricle could be clearly determined in all rats. The distance between the middle of the third ventricle and the outer border of both hemispheres (distance from ipsilateral border to third ventricle: *A* and distance from contralateral border to third ventricle: *B*) was measured [17] and MLS was calculated by the following equation:

$$MLS = (A - B)/2 \quad (2)$$

2.7.3 T2RT

For quantification of the T2RT, we used Bruker's implemented image processing tool. On the six contiguous coronal slices, regions of interest (ROIs) were set in the center of the ischemic lesions in the cortex and subcortex and on the corresponding position of the contralateral hemisphere, and the side-to-side differences of the T2RT were calculated.

2.8 Postmortem analysis: quantification of BWC by the wet–dry technique

After MRI, the animals were deeply anesthetized and decapitated. The brains were removed and separated into the ipsi- and contralateral hemispheres. The wet weight of each hemisphere was measured, then the tissue was dried to a constant weight at 50°C and weighed again (dry weight). The absolute BWC ($\%H_2O$) was calculated as follows [18]:

$$\%H_2O = ([\text{wet weight} - \text{dry weight}]/\text{wet weight} \times 100) \quad (3)$$

Equation (4) was used to calculate the increase in BWC in the ipsilateral hemisphere compared to the unaffected contralateral hemisphere ($\%\Delta H_2O$) [18]:

$$\%\Delta H_2O = \%H_2O_{ipsilateral} - \%H_2O_{contralateral} \quad (4)$$

2.9 fpVCT

The CBF was quantified after the 24-h clinical testing with an fpVCT, which was developed by GE Healthcare, London, Ontario, Canada. The system is described in detail in the study by Obert et al. (2010) [31]. Preparation and anesthesia of the rats, image acquisition, reconstruction, and analysis followed a previously published protocol [32].

2.10 Placing the ROIs, infarct core, and hemisphere

Since the infarcted brain regions cannot be properly displayed on perfusion slices, the corresponding 2,3,5-triphenyltetrazolium chloride (TTC)-stained slices were used to identify the extent and location of ischemic areas. After VCT investigation, the animals were deeply anesthetized using isoflurane and euthanized by decapitation; the brains were removed and sectioned

coronally into six slices (thickness: 2 mm each), incubated in a 2% solution of TTC at 37°C, fixed by immersion in 10% buffered formalin solution, and scanned with a computer scanner (ScanJet 3400C; Hewlett Packard; resolution 600 × 600 dpi). The unstained areas of the fixed brain slices were defined as the ischemic infarction.

For ipsi- and contralateral sides, flexibly created freehand ROIs included cortical and subcortical regions of the infarct core as well as the whole hemisphere. The CBF (ml/100 g/min) was acquired as mean for each side or the corresponding region and in each animal, thus permitting comparison of data between infarct hemisphere and non-infarct hemisphere. Differences in CBF were calculated by the following equation:

$$\%\text{CBF difference} = (\text{mean CBF ipsilateral}/\text{mean CBF contralateral}) \times 100 \quad (5)$$

2.11 Statistical analysis

The Shapiro–Wilk test was used to test for normal distribution of parametric data. Homogeneity of variance was tested by the Levene test. Erythropoietin treatment and placebo groups were compared separately for craniectomy and sham craniectomy by unpaired Student's t test or, for data not passing the normality test, the nonparametric Mann–Whitney U test. Data are presented as mean ± standard deviation. The level of probability $p < 0.05$ was regarded as significant (SPSS v21; IBM, Germany).

3 Results

Twenty-six animals had to be excluded from this study: for seven animals, technical problems occurred during contrast agent infusion, and the imaging of another four rats was hampered due to motion artifacts. Seven animals suffered cerebral hemorrhage, six animals died during craniectomy, and two rats showed no ischemic infarction. The remaining 84 animals completed the study protocol.

Pre-MCAO Rotarod performance, body weight, and body temperature did not differ significantly between the groups.

3.1 Neurological impairment

The results in Rotarod test performance pre- vs. 24 h post-MCAO did not differ significantly between the craniectomy (5.71 ± 11.14 rpm vs. 6.29 ± 9.95 rpm; $p = 0.837$) and sham craniectomy groups (5.43 ± 11.21 rpm vs. 6.00 ± 7.10 rpm; $p = 0.786$).

3.2 Infarct size

Measurement by MRI of infarct sizes corrected for the space-occupying effect of brain edema revealed no difference ($t(18) = 1.391$; $p = 0.181$, $d = 0.62$) between craniectomy rats receiving placebo (36.29 ± 10.21%) vs. EPO (30.34 ± 8.86%). The mean ischemic lesion volume after 24 h was significantly smaller ($t(18) = 2.497$; $p = 0.022$, $d = 1.12$) in EPO-treated animals without craniectomy (25.28 ± 7.03%) when compared to placebo animals without craniectomy (34.83 ± 9.84%) (Figure 1).

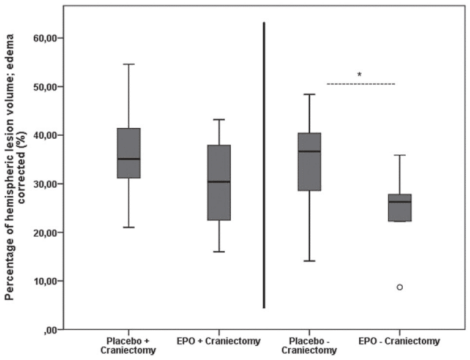

Figure 1: Mean ischemic lesion volume as determined on MRI, expressed in percentage of hemispheric volume (%HLV_{ec}). Significantly reduced mean ischemic lesion volume for EPO-treated animals compared to the placebo group without craniectomy (*$p = 0.022$; t-test). No significant difference could be detected between craniectomy groups. Outliers are marked with a circle (out values) or a star (far out values).

3.3 Brain edema

The MLS of the animals treated with EPO (0.083 ± 0.027 cm), which were not craniectomized, was significantly reduced ($t(18) = 2.768$; $p = 0.013$; $d = 1.24$) when

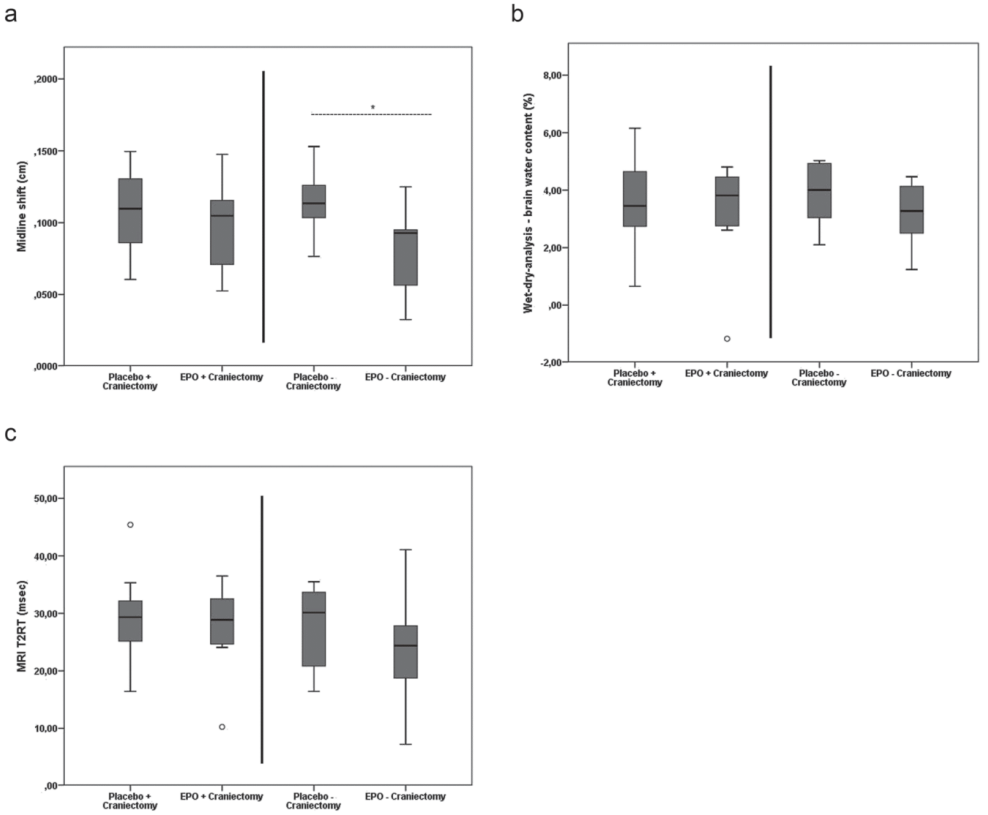

Figure 2: MLS (a), BWC (b), and T2RT (c). (a) MLS in the groups without craniectomy was significantly smaller in animals treated with EPO when compared to the placebo animals (*$p = 0.013$). No significant differences were observed within the craniectomy groups. (b) BWC and (c) T2RT displayed no significant differences between the groups. Outliers are marked with a circle (out values) or a star (far out values).

compared to the noncraniectomized placebo animals (0.114 ± 0.023 cm). This could not be observed in the two craniectomy groups (placebo: 0.109 ± 0.029 cm vs. EPO: 0.100 ± 0.031 cm, respectively; $t(18) = 0.613$; $p = 0.548$; $d = 0.30$) (Figure 2a).

In the EPO- and placebo-treated craniectomy groups, BWC (placebo: 3.33 ± 1.75% vs. EPO: 3.48 ± 1.61%, respectively; $z = 1.71$; $p = 0.912$; $d = 0.09$) and T2RT (placebo: 29.50 ± 7.79 ms vs. EPO: 27.57 ± 7.39 ms, respectively; $t(18) = 0.569$; $p = 0.576$; $d = 0.25$) showed no significant difference. A similar result could be obtained from the analyses of BWC (placebo: 3.87 ± 1.02% vs. EPO: 3.19 ± 1.11%; $z = -1.21$; $p = 0.247$; $d = 0.64$) and T2RT (placebo: 28.25 ± 6.65 ms vs. EPO: 23.54 ± 9.14 ms; $t(18) = 1.318$; $p = 0.204$; $d = 0.59$) in noncraniectomy groups III + IV (Figure 2b and c).

3.4 CBF

CBF was acquired within cortical and subcortical regions of the infarct core as well as the whole hemisphere and expressed as a ratio between the ipsilateral and the contralateral sides.

In the absence of craniectomy (groups III + IV), the EPO-treated animals showed a significant increase in CBF in cortical regions of the infract core when compared to

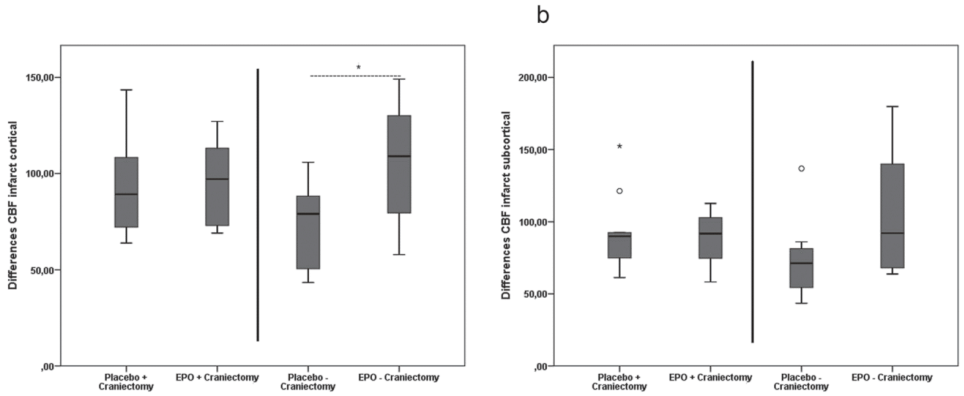

Figure 3: CBF in the infarct core. There was a significant CBF increase with EPO treatment in the cortical regions of the infarct core when compared to placebo treatment in the absence of craniectomy (*p = 0.043 u-test) (a). No significant CBF changes could be observed in the cortical regions of the infarct core when craniectomy was performed (a) or in subcortical regions (b). Outliers are marked with a circle (out values) or a star (far out values).

the placebo-treated animals (placebo: 72.29 ± 24.00% vs. EPO: 105.53 ± 33.10%, respectively; $t(18) = -2.245$; $p = 0.043$; $d = 1.00$) (Figure 3a). In the subcortical regions (placebo: 74.29 ± 29.04 vs. EPO: 103.38 ± 39.54%, respectively; $t(18) = -1.799$; $p = 0.091$; $d = 0.85$) and the total infarct core (placebo: 76.58 ± 28.03 vs. EPO: 104.48 ± 33.49%, respectively; $t(18) = -1.975$; $p = 0.065$; $d = 0.90$), tendencies did not reach statistical significance. No significant difference in CBF was observed in the cortical (placebo: 94.40 ± 28.62% vs. EPO: 95.12 ± 23.79%, respectively; $t(18) = -0.05$; $p = 0.961$; $d = 0.03$) or subcortical region (placebo: 88.94 ± 19.00% vs. EPO: 92.74 ± 26.73%, respectively; $t(18) = 0.378$; $p = 0.709$; $d = 0.16$) or the whole infarct core within the craniectomy groups (placebo: 90.89 ± 20.39% vs. EPO: 96.67 ± 28.19%, respectively; $t(18) = 0.542$; $p = 0.594$; $d = 0.23$) (groups I + II, Figure 3).

Between the craniectomy groups, investigation of hemispherical blood flow in the cortical (placebo: 108.59 ± 19.46% vs. EPO: 103.75 ± 11.68%, respectively; $t(18) = 0.706$; $p = 0.488$; $d = 0.30$) and subcortical regions (placebo: 96.94 ± 7.15% vs. EPO: 94.11 ± 11.30%, respectively; $t(18) = 0.699$; $p = 0.492$; $d = 0.29$) and total hemisphere (placebo: 103.70 ± 15.81% vs. EPO: 100.13 ± 10.96%, respectively; $t(18) = 0.616$; $p = 0.545$; $d = 0.26$) revealed no significant effects of EPO treatment. Similar results were shown in the comparison of CBF between groups III + IV without craniectomy in the cortical (placebo: 91.25 ± 16.45% vs. EPO: 99.12 ± 18.04%, respectively; $t(18) = -1.020$; $p = 0.312$; $d = 0.46$) and subcortical regions (placebo: 90.02 ± 11.54% vs. EPO: 90.43 ± 9.18%, respectively; $t(18) = -0.087$; $p = 0.931$; $d = 0.04$) and total hemisphere (placebo: 91.26 ± 11.86% vs. EPO: 94.36 ± 10.90%, respectively; $t(18) = -0.609$; $p = 0.550$; $d = 0.27$) (Figure 4).

5 Discussion

Investigations on rodent stroke models [16–18,33] indicate that the development of vasogenic brain edema within the hyperacute phase of stroke (<6 h) may exert a significant, possibly underestimated, influence on the progression of ischemic area, as swelling of ischemic tissue within the fixed cranial volume can lead to impairment of microcirculation in the critically hypoperfused penumbral area. Hence, collateral damage caused by the space-occupying effect of a large MCA territory stroke accounts for up to 50% of ischemic lesion formation [16]. Therapeutic measures aiming to reduce cerebral edema and its resulting space-occupying effect in the early stages of stroke may operate as indirect or "secondary" neuroprotectants [16,29]. Investigations on the effects of systemically administered rhEPO prior to transient MCAO in rodents suggest that neuroprotection results more from the mitigation of brain edema than from direct antiapoptotic effects on neurons [27]. We hypothesized that EPO administered prior to transient MCAO exerts its neuroprotective properties in the early phase of stroke primarily via secondary neuroprotection by reduction of cerebral edema.

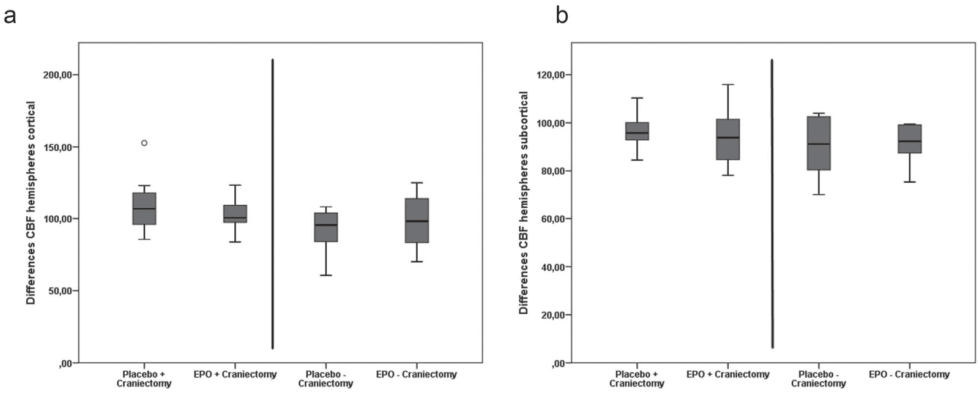

Figure 4: CBF in the hemisphere. The CBF measurement of the whole hemispheres, i.e., cortical (a) and subcortical (b) regions, revealed no significant effects of EPO treatment regardless of craniectomy. Outliers are marked with a circle (out values).

Craniectomy has been shown to save the lives of patients with large space-occupying territorial strokes in severe danger of cerebral herniation and death and was proven in large clinical trials to reduce mortality significantly, from 71 to 22% [34,35]. Experimental studies on the effect of craniectomy in a rodent model of MCAO report a significant reduction of infarct size, mainly attributed to the release of mechanical compression [36,37]. To approach the distinction of primary from secondary neuroprotection in our study, elimination of increased intracranial pressure due to the space-occupying ischemia was achieved by bilateral craniectomy prior to transient MCAO [16,29]. Thus, the preponderance of edema reduction via EPO was expected to lead to pronounced group differences regarding infarct size and edema volume, which are dependent on integrity of the skull.

We observed that rhEPO treatment before transient MCAO reduced edema-corrected infarction size by approximately 10%. Data on experiments with a comparable setting are limited; two previous studies on rats reported no significant effects on infarct volume for EPO pretreatment. In contrast, a study on mice showed infarct reduction up to 47% [27,38,39]. Interestingly, in the present investigation, a significant reduction compared to placebo treatment could only be observed if the skull was left intact; an approximation of infarct sizes could be quantified with craniectomy.

Experimental research on rodent stroke models provides robust evidence for the antiedematic effects of EPO, which has particularly been attributed to a preserved barrier function of the BBB [40–45]. An investigation on the markers of BBB integrity – such as occludin, alpha-, and beta-catenin – demonstrated that EPO treatment before and 3 days after focal cerebral ischemia can stabilize the BBB, reduce its permeability, and thereby control cerebral inflammation and edema [43]. Impermeability of the BBB mainly depends on intact endothelial cells and tight junctions, which are subjected to substantial oxidative stress by generation of reactive oxygen species and lipid peroxidation during the phase of reperfusion after transient ischemia [46,47]. Under this condition, EPO seems to stimulate endothelial nitric oxide production and has the ability to prevent reperfusion-mediated injury to the BBB [48]. Due to the fact that lesion volume is proportional to hemispheric BWC, the volume of infarcted tissue can bias methods for quantification of BWC that include whole hemispheres, such as the wet–dry technique and determination of MLS; cerebral edema in the present investigation was therefore assessed on MRI using T2RT measurements in ROIs, since this method was shown to be largely independent of lesion size [18]. We could demonstrate a significant reduction in MLS for EPO pretreatment only in the absence of bilateral craniectomy. In this group, MRI T2RT presented a trend toward the lowest mean values for the treatment group with intact skull but missed statistical significance. Nevertheless, these data seem to suggest that neuroprotection of EPO pretreatment in transient MCAO implies a strong antiedematic effect.

We used fpVCT for noninvasive dynamic imaging of cerebral perfusion after temporary MCAO in the cortical and subcortical regions of the infarct and the whole hemisphere [32]. Without craniectomy, the EPO

pretreatment led to a significant increase in CBF in the cortical regions of the ischemic tissue. However, in subcortical areas of the infarct and the whole hemispheres, no significant alterations of CBF could be objectified. Xiong et al. described EPO neuroprotection after traumatic brain injury even in EpoR null mice and attributed this effect particularly to vascular protection [49]. Li et al. investigated angiogenesis in mice that received rhEPO 30 minutes before and once daily after ischemic stroke and observed enhanced angiogenic activity between days 7 and 21; on day 14, the CBF reached preischemia initial values [50]. Furthermore, in a rabbit model for subarachnoid hemorrhage, intravenously administered rhEPO led to a significantly increased CBF between days 2 and 16 [51]. In addition to these observations of EPO's time-sensitive effects, Shafi et al. used isolated rat MCA to demonstrate that luminal-applied EPO can directly dilate arteries and that 24-h pretreatment with EPO potentiates this effect [52]; after this single-dose pretreatment with EPO and transient MCAO, we only observed a significant increase in CBF in the defined cortical regions of the infarct and in the absence of craniectomy. If compression on the brain, microvasculature and presumable pial and venous vessels is released by craniectomy, the CBF in EPO- and placebo-treated rats is equal. This seems to display a local effect for the defined area of the ischemia, as no differences in CBF could be observed for total hemispheres regardless of the EPO treatment or craniectomy. A focal improvement in CBF in the cortical regions of the ischemic area may indicate a more efficient collateralization with EPO, either via its antiedemic and pressure-reducing mode of action or due to its direct vasodilatative effects. Improved collateralization in turn supports the recovery of critically perfused penumbral areas reducing the infarct core, which has been shown by a significant reduction in ischemic lesion volume. In line with the aforementioned data on infarct size and edema reduction, the latter could only be objectified in the absence of craniectomy, i.e., a situation in which pressure variations are supposed to be the most pronounced.

The results of this study have to be interpreted with caution, as surrogate parameters for a secondary neuroprotective mechanism of action were considered. These can be regarded as hypothesis generating but must subsequently be confirmed in the corresponding mechanistic studies to objectively distinguish a direct or indirect mechanism of action.

In this study, rhEPO was administered – a compound that, because of its low BBB permeability, must be applied in comparatively high intravenous doses, prompting several dose-dependent side effects such as increased hematocrit and hypertension as well as procoagulatory and prothrombotic effects on microcirculation. These side effects seem to be primarily due to the erythropoietic mode of action of the EPO derivate, and it is conceivable in principle that they limit the extent of neuroprotection in the context of acute cerebrovascular diseases. Therefore, efforts have been made in the past to support EPO-mediated cytoprotection without affecting the hematopoietic system. In this respect, it could be shown that, putatively due to an altered receptor interaction, carbamylated EPO and mutants such as EPO-S100E or EPO-R103E act neuroprotectively but lack erythropoietic activity with a drastically reduced $(EPOR)_2$ affinity. In addition, the fusion protein EPO-Tat possesses a significantly enhanced BBB permeability and thus enables the use of lower effective doses [53,54]. It therefore remains to be discussed whether the use of another EPO derivative yielded different, clearer results.

Another aspect of pharmacokinetics appears to be of particular interest in connection with the application of EPO prior to MCAO. In a rodent model of traumatic brain injury, it was shown not only that EPO must be administered in high doses when applied peripherally and that intravenous is superior to intraperitoneal administration but also that rhEPO crosses the BBB with a delay of approximately 4 h and appears to develop its biological effect after around 8 h [1]. Moreover, the half-life of rhEPO after single injection was reported to be between 25.6 h and 35.5 h [2]. If this time frame of pharmacokinetics and the edema dynamics after cerebral infarction with the onset immediately after ischemia are taken into account, an even earlier time of application of EPO could possibly have led to a more pronounced neuroprotective effect. Thus, the single administration of EPO immediately after the onset of ischemia, which is more similar to the clinical situation in stroke, is not expected to produce significantly different results. As described in the introduction, the timing was chosen against the background of anticipatory neuroprotection in cerebrovascular interventions, for example, where the onset of damage is known; a transfer of the results to acute stroke therapy is only possible with difficulty.

With respect to the effect sizes of infarct volumes and perfusion parameters, although the number of animals per group seems to be sufficient, it remains debatable whether larger groups for investigations on brain edema would have led to significantly different results.

Clinical testing did not point out a statistically significant functional improvement, which might indicate limited sensitivity of the clinical tests in general or with regard to the chronological parameters and points in time selected in this study. Furthermore, the study design does not allow quantification of possible long-term improvement. In principle, the use of healthy animals, controlled laboratory conditions, and application of anesthesia can hamper the assignability of findings from bench to bedside, which has to be considered when findings are interpreted.

6 Conclusion

Interventions for predictable stroke risk substantiate discussion on anticipatory neuroprotection preceding the risk-related procedure and intended for prevention of neuronal loss. This study demonstrates that a single dose of rhEPO 5,000 IU/kg given prior to transient MCAO in rats significantly reduces ischemic lesion volume, decreases MLS, and increases local CBF in the cortical regions of ischemia after 24 h. Data may suggest an interaction between edema and pressure reducing as well as blood flow-increasing effects mediated by EPO.

Acknowledgments: This work was generously supported by the Willy Robert Pitzer Foundation (AZ8006) and by the Kerckhoff Clinic Bad Nauheim.

Conflict of interest: The authors states no conflict of interest.

References

[1] Lieutaud T, Andrews PJD, Rhodes JKJ, Williamson R. Characterization of the pharmacokinetics of human recombinant erythropoietin in blood and brain when administered immediately after lateral fluid percussion brain injury and its pharmacodynamic effects on IL-1beta and MIP-2 in rats. J Neurotrauma. 2008;25:1179–85.

[2] Xenocostas A, Cheung WK, Farrell F, Zakszewski C, Kelley M, Lutynski A, et al. The pharmacokinetics of erythropoietin in the cerebrospinal fluid after intravenous administration of recombinant human erythropoietin. Eur J Clin Pharmacol. 2005;61:189–95.

[3] Sargin D, Friedrichs H, El-Kordi A, Ehrenreich H. Erythropoietin as neuroprotective and neuroregenerative treatment strategy: comprehensive overview of 12 years of preclinical and clinical research. Best Pract Res Clin Anaesthesiol. 2010;24:573–94.

[4] Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, et al. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. Proc Natl Acad Sci USA. 2000;97:10526–31.

[5] Minnerup J, Heidrich J, Rogalewski A, Schäbitz W-R, Wellmann J. The efficacy of erythropoietin and its analogues in animal stroke models: a meta-analysis. Stroke. 2009;40:3113–20.

[6] Jerndal M, Forsberg K, Sena ES, Macleod MR, O'Collins VE, Linden T, et al. A systematic review and meta-analysis of erythropoietin in experimental stroke. J Cereb Blood Flow Metab. 2010;30:961–8.

[7] van der Kooij MA, Groenendaal F, Kavelaars A, Heijnen CJ, van Bel F. Neuroprotective properties and mechanisms of erythropoietin in in vitro and in vivo experimental models for hypoxia/ischemia. Brain Res Rev. 2008;59:22–33.

[8] Noguchi CT, Asavaritikrai P, Teng R, Jia Y. Role of erythropoietin in the brain. Crit Rev Oncol Hematol. 2007;64:159–71.

[9] Minnerup J, Wersching H, Schäbitz W-R. EPO for stroke therapy - Is there a future for further clinical development? Exp Transl Stroke Med. 2010;2:10.

[10] Sirén AL, Knerlich F, Poser W, Gleiter CH, Brück W, Ehrenreich H. Erythropoietin and erythropoietin receptor in human ischemic/hypoxic brain. Acta Neuropathol. 2001;101:271–6.

[11] Sola A, Wen T-C, Hamrick SEG, Ferriero DM. Potential for protection and repair following injury to the developing brain: a role for erythropoietin? Pediatr Res. 2005;57:110R–7R.

[12] Minnerup J, Schäbitz W-R. Multifunctional actions of approved and candidate stroke drugs. Neurotherapeutics. 2009;6:43–52.

[13] Brines M, Grasso G, Fiordaliso F, Sfacteria A, Ghezzi P, Fratelli M, et al. Erythropoietin mediates tissue protection through an erythropoietin and common beta-subunit heteroreceptor. Proc Natl Acad Sci USA. 2004;101:14907–12.

[14] Sirén AL, Ehrenreich H. Erythropoietin – a novel concept for neuroprotection. Eur Arch Psychiatry Clin Neurosci. 2001;251:179–84.

[15] Gerriets T, Stolz E, Walberer M, Müller C, Rottger C, Kluge A, et al. Complications and pitfalls in rat stroke models for middle cerebral artery occlusion: a comparison between the suture and the macrosphere model using magnetic resonance angiography. Stroke. 2004;35:2372–7.

[16] Walberer M, Ritschel N, Nedelmann M, Volk K, Mueller C, Tschernatsch M, et al. Aggravation of infarct formation by brain swelling in a large territorial stroke: a target for neuroprotection? J Neurosurg. 2008;109:287–93.

[17] Walberer M, Blaes F, Stolz E, Müller C, Schoenburg M, Tschernatsch M, et al. Midline-shift corresponds to the amount of brain edema early after hemispheric stroke – an MRI study in rats. J Neurosurg Anesthesiol. 2007;19:105–10.

[18] Gerriets T, Stolz E, Walberer M, Müller C, Kluge A, Bachmann A, et al. Noninvasive quantification of brain edema and the space-occupying effect in rat stroke models using magnetic resonance imaging. Stroke. 2004;35:566–71.

[19] Daou B, Chalouhi N, Starke RM, Barros G, Ya'qoub L, Do J, et al. Clipping of previously coiled cerebral aneurysms: efficacy, safety, and predictors in a cohort of 111 patients. J Neurosurg. 2016;125:1337–43.

[20] Ha S-K, Lim D-J, Seok B-G, Kim S-H, Park J-Y, Chung Y-G. Risk of stroke with temporary arterial occlusion in patients

undergoing craniotomy for cerebral aneurysm. J Korean Neurosurg Soc. 2009;46:31–7.
[21] Goyal M, Menon BK, van Zwam WH, Dippel DWJ, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. Lancet. 2016;387:1723–31.
[22] Gill HL, Siracuse JJ, Parrack I-K, Huang ZS, Meltzer AJ. Complications of the endovascular management of acute ischemic stroke. Vasc Health Risk Manag. 2014;10:675–81.
[23] Palmerini T, Biondi-Zoccai G, Della Riva D, Mariani A, Savini C, Di Eusanio M, et al. Risk of stroke with percutaneous coronary intervention compared with on-pump and off-pump coronary artery bypass graft surgery: Evidence from a comprehensive network meta-analysis. Am Heart J. 2013;165:910–7.e14.
[24] Smit Y, Vlayen J, Koppenaal H, Eefting F, Kappetein AP, Mariani MA. Percutaneous coronary invervention versus coronary artery bypass grafting: a meta-analysis. J Thorac Cardiovasc Surg. 2015;149:831-8.e1-13.
[25] Mao Z, Zhong X, Yin J, Zhao Z, Hu X, Hackett ML. Predictors associated with stroke after coronary artery bypass grafting: a systematic review. J Neurol Sci. 2015;357:1–7.
[26] Calvet D, Mas J-L. Recent advances in carotid angioplasty and stenting. Int J Stroke. 2016;11:19–27.
[27] Ratilal BO, Arroja MMC, Rocha JPF, Fernandes AMA, Barateiro AP, Brites DMTO, et al. Neuroprotective effects of erythropoietin pretreatment in a rodent model of transient middle cerebral artery occlusion. J Neurosurg. 2014;121:55–62.
[28] Wang L, Zhang Z, Wang Y, Zhang R, Chopp M. Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in rats. Stroke. 2004;35:1732–7.
[29] Walberer M, Tschernatsch M, Fischer S, Ritschel N, Volk K, Friedrich C, et al. RNase therapy assessed by magnetic resonance imaging reduces cerebral edema and infarction size in acute stroke. Curr Neurovasc Res. 2009;6:12–9.
[30] Hamm RJ, Pike BR, O'Dell DM, Lyeth BG, Jenkins LW. The rotarod test: an evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. J Neurotrauma. 1994;11:187–96.
[31] Obert M, Schulte-Geers C, Schilling RL, Harth S, Kläver M, Traupe H, et al. High-resolution flat-panel volumetric CT images show no correlation between human age and sagittal suture obliteration – independent of sex. Forensic Sci Int. 2010;200:180.e1-12.
[32] Juenemann M, Goegel S, Obert M, Schleicher N, Ritschel N, Doenges S, et al. Flat-panel volumetric computed tomography in cerebral perfusion: evaluation of three rat stroke models. J Neurosci Methods. 2013;219:113–23.
[33] Gerriets T, Stolz E, Walberer M, Müller C, Kluge Al, Kaps M, et al. Middle cerebral artery occlusion during MR-imaging: investigation of the hyperacute phase of stroke using a new in-bore occlusion model in rats. Brain Res Brain Res Protoc. 2004;12:137–43.
[34] Vahedi K, Hofmeijer J, Juettler E, Vicaut E, George B, Algra A, et al. Early decompressive surgery in malignant infarction of the middle cerebral artery: a pooled analysis of three randomised controlled trials. Lancet Neurol. 2007;6:215–22.
[35] Schwab S, Steiner T, Aschoff A, Schwarz S, Steiner HH, Jansen O, et al. Early hemicraniectomy in patients with complete middle cerebral artery infarction. Stroke. 1998;29:1888–93.
[36] Engelhorn T, von Kummer R, Reith W, Forsting M, Doerfler A. What is effective in malignant middle cerebral artery infarction: reperfusion, craniectomy, or both? An experimental study in rats. Stroke. 2002;33:617–22.
[37] Hofmeijer J, Schepers J, Veldhuis WB, Nicolay K, Kappelle LJ, Bär PR, et al. Delayed decompressive surgery increases apparent diffusion coefficient and improves peri-infarct perfusion in rats with space-occupying cerebral infarction. Stroke. 2004;35:1476–81.
[38] Bernaudin M, Marti HH, Roussel S, Divoux D, Nouvelot A, MacKenzie ET, et al. A potential role for erythropoietin in focal permanent cerebral ischemia in mice. J Cereb Blood Flow Metab. 1999;19:643–51.
[39] Dahlberg SA, Xu L, Hess DC, Hohnadel E, Hill WD, Fagan SC. Erythropoietin and Erythropoietin Mimetic Peptide in Focal Cerebral Ischemia. Stroke. 2004;35:279.
[40] Wang R, Wu X, Liang J, Qi Z, Liu X, Min L, et al. Intra-artery infusion of recombinant human erythropoietin reduces blood-brain barrier disruption in rats following cerebral ischemia and reperfusion. Int J Neurosci. 2015;125:693–702.
[41] Chi OZ, Hunter C, Liu X, Weiss HR. Effects of erythropoietin on blood-brain barrier disruption in focal cerebral ischemia. Pharmacology. 2008;82:38–42.
[42] Zechariah A, ElAli A, Hermann DM. Combination of tissue-plasminogen activator with erythropoietin induces blood-brain barrier permeability, extracellular matrix disaggregation, and DNA fragmentation after focal cerebral ischemia in mice. Stroke. 2010;41:1008–12.
[43] Li Y, Lu Z-Y, Ogle M, Wei L. Erythropoietin prevents blood brain barrier damage induced by focal cerebral ischemia in mice. Neurochem Res. 2007;32:2132–41.
[44] Gunnarson E, Song Y, Kowalewski JM, Brismar H, Brines M, Cerami A, et al. Erythropoietin modulation of astrocyte water permeability as a component of neuroprotection. Proc Natl Acad Sci USA. 2009;106:1602–7.
[45] Calapai G, Marciano MC, Corica F, Allegra A, Parisi A, Frisina N, et al. Erythropoietin protects against brain ischemic injury by inhibition of nitric oxide formation. Eur J Pharmacol. 2000;401:349–56.
[46] Blasig IE, Mertsch K, Haseloff RF. Nitronyl nitroxides, a novel group of protective agents against oxidative stress in endothelial cells forming the blood-brain barrier. Neuropharmacology. 2002;43:1006–14.
[47] Bahcekapili N, Uzüm G, Gökkusu C, Kuru A, Ziylan Y. The relationship between erythropoietin pretreatment with blood-brain barrier and lipid peroxidation after ischemia/reperfusion in rats. Life Sci. 2007;80:1245–51.
[48] Utepbergenov DI, Mertsch K, Sporbert A, Tenz K, Paul M, Haseloff RF, et al. Nitric oxide protects blood-brain barrier in vitro from hypoxia/reoxygenation-mediated injury. FEBS Lett. 1998;424:197–201.
[49] Xiong Y, Mahmood A, Qu C, Kazmi H, Zhang ZG, Noguchi CT, et al. Erythropoietin improves histological and functional outcomes after traumatic brain injury in mice in the absence of the neural erythropoietin receptor. J Neurotrauma. 2010;27:205–15.
[50] Li Y, Lu Z, Keogh CL, Yu SP, Wei L. Erythropoietin-induced neurovascular protection, angiogenesis, and cerebral blood flow restoration after focal ischemia in mice. J Cereb Blood Flow Metab. 2007;27:1043–54.

[51] Murphy AM, Xenocostas A, Pakkiri P, Lee T-Y. Hemodynamic effects of recombinant human erythropoietin on the central nervous system after subarachnoid hemorrhage: reduction of microcirculatory impairment and functional deficits in a rabbit model. J Neurosurg. 2008;109:1155–64.
[52] Shafi NI, Andresen J, Marrelli SP, Bryan RM. Erythropoietin potentiates EDHF-mediated dilations in rat middle cerebral arteries. J Neurotrauma. 2008;25:257–65.
[53] Zhang F, Xing J, Liou AK-F, Wang S, Gan Yu, Luo Y, et al. Enhanced delivery of erythropoietin across the blood-brain barrier for neuroprotection against ischemic neuronal injury. Transl Stroke Res. 2010;1:113–21.
[54] Leist M, Ghezzi P, Grasso G, Bianchi R, Villa P, Fratelli M, et al. Derivatives of erythropoietin that are tissue protective but not erythropoietic. Science. 2004;305: 239–42.

5.3. Microbubble-mediated Sonothrombolysis with BR38 of a Venous Full Blood Thrombus in a Rat Embolic Stroke Model

Tobias Braun*, Laura Sünner*, Maaike Hachenberger, Clemens Müller, Astrid Wietelmann, Martin Juenemann, Jörn Pons-Kühnemann, Manfred Kaps, Tibo Gerriets, Marlene Tschernatsch, Joachim Roth, Mesut Yenigün

*contributed equally

Beim ischämischen Schlaganfall geht die frühe Wiedereröffnung eines verschlossenen Gefäßes mit einem besseren klinisch-funktionellen Outcome einher. Die systemische intravenöse Lyse mit rekombinantem Gewebsplasminogen-Aktivator (rt-PA) ist jedoch nur bei einer Minderheit der Patienten möglich. Auch ist es hierdurch, insbesondere bei proximalen Verschlüssen großer Gefäße, oft nicht möglich, das verschlossene Gefäß wieder zu eröffnen. Die mechanische Rekanalisation (= Thrombektomie) ist hierfür erfolgsversprechender, jedoch kann sie nur bei ausgewählten Patienten mit proximalen Gefäßverschlüssen erfolgen. Auch steht die Thrombektomie nur in spezialisierten Zentren zur Verfügung und macht gegebenenfalls den Transport in ein solches Zentrum notwendig. Daher stellt die Methode der Sonothrombolyse eine mögliche alternative oder komplementäre Behandlungsmöglichkeit dar. Wir untersuchten in dieser Studie eine ultraschallkontrastmittel-unterstütze Sonothrombolyse (mmSTL) in einem thrombembolischen Hirninfarktmodell mit Verschluss der A. cerebri media (MCAO) bei Ratten.

Hierfür wurde bei 67 männlichen Wistar-Ratten eine MCAO mittels eines autologen Vollblutthrombus durchgeführt. Die Tiere wurden in vier Behandlungsgruppen randomisiert und erhielten entweder rt-PA, mmSTL, die Kombination beider Verfahren oder eine Placebobehandlung. Die durchgeführten Untersuchungen umfassten eine neurologische Untersuchung, die Messung der Infarktgröße und die Untersuchung auf intrazerebrale Blutungen in einer MRT-Bildgebung sowie eine histologische Untersuchung auf das Vorhandensein von Mikroblutungen.

In der neurologischen Untersuchung 24 Stunden nach Infarktinduktion zeigten sich keine Unterschiede zwischen den Behandlungsgruppen. In allen Behandlungsarmen lag eine Reduktion der Infarktgröße 24 Stunden nach Infarktinduktion verglichen zu Placebo vor ($p \leq 0,05$). Jedoch lagen keine Unterschiede zwischen den aktiven Behandlungsarmen vor ($p > 0.05$) (Placebo 0,75 cm3 ± 0,10 cm3; mmSTL 0,43 cm3 ± 0,07 cm3; rt-PA 0,4 cm3 ± 0,07 cm3; mmSTL + rt-PA 0,27 cm3 ± 0,08 cm3). Histologisch zeigten sich bei allen Tieren intrazerebrale Mikroblutungen. Die Häufigkeit größerer Blutungen, die mittels MRT-Bildgebung darstellbar waren, unterschied sich nicht innerhalb der Gruppen (Placebo 3; mmSTL 4; rt-PA 2; mmSTL

+ rt-PA 2; $p > 0,05$) und ging auch nicht mit einer geringeren Leistung in der klinischen Testung einher. Es lagen keine Unterschiede bezüglich der Mortalität innerhalb der Gruppen vor ($p > 0,05$).

Zusammenfassend zeigte unsere Studie, dass eine mmSTL in Kombination mit rt-PA oder ohne eine effektive und sichere Behandlungsmethode im embolischen Hirninfarktmodell mit autologen Vollblutthrombus bei der Ratte darstellt. Die Sonothrombolyse könnte damit eine Behandlungsmethode bei Patienten mit distal gelegenen Gefäßverschlüssen darstellen. Auch könnte sie als überbrückende Therapie bei einem notwendigen Transport in ein Thrombektomiezentrum angewandt werden.

Original Article

Microbubble-Mediated Sonothrombolysis with BR38 of a Venous Full Blood Thrombus in a Rat Embolic Stroke Model

Tobias Braun[1,2*], Laura Sünner[2*], Maaike Hachenberger[1,2], Clemens Müller[3], Astrid Wietelmann[4], Martin Juenemann[1,2], Jörn Pons-Kühnemann[5], Manfred Kaps[1], Tibo Gerriets[1,2,6], Marlene Tschernatsch[1,2,6], Joachim Roth[7], Mesut Yenigün[1,2]

*both authors contributed equally to this manuscript

[1] Department of Neurology, Faculty of Medicine, Justus-Liebig-University, Klinikstrasse 33, D-35392 Giessen, Germany

[2] Heart & Brain Research Group, Justus-Liebig-University Giessen and Kerckhoff Clinic, Benekestrasse 2-8, 61231 Bad Nauheim, Germany

[3] Department of Radiology, Kerckhoff Clinic, Benekestrasse 2-8, 61231 Bad Nauheim, Germany

[4] Scientific Service Group Magnetic Resonance Imaging, Max-Planck-Institute for Heart and Lung Research, Ludwigstraße 43, 61231 Bad Nauheim, Germany.

[5] Institute of Medical Informatics, Department of medical Statistics, Justus Liebig University Giessen, Giessen, Germany.

[6] Department of Neurology, Gesundheitszentrum Wetterau, Chaumontplatz 1, D-61231 Bad Nauheim, Germany

[7] Department of veterinarian physiology and biochemistry, Justus-Liebig-University Giessen, Frankfurter Strasse 100, 35392 Giessen

Corresponding author:

Tobias Braun, MD

Department of Neurology, Justus-Liebig-University

Klinikstraße 33

D-35385 Giessen, Germany

Phone: +49 641 98556827

Fax: +49 641 98545359

E-mail address: tobias.braun@neuro.med.uni-giessen.de

Running title: Sonothrombolysis with BR38 in experimental embolic stroke.

Author contributions:

(I) Conception and design: TB, MY, TG

(II) Administrative support: MK, TG, AW, CM

(III) Provision of study materials or patients: AW, CM

(IV) Collection and assembly of data: LS, MT, MS, MJ

(V) Data analysis and interpretation: TB, LS, J P-K, MY, JR

(VI) Manuscript writing: All authors

(VII) Final approval of manuscript: All authors

Abstract

Background

Early recanalization of an occluded vessel is associated with a better clinical outcome in acute ischemic stroke. Intravenous thrombolysis using recombinant tissue plasminogen activator (rt-PA) is only available in a minority of patients and often fails to reopen the occluded vessel. Mechanical recanalization is more effective in this matter but only available for selected patients when a thrombectomy centre can be reached. Therefore, sonothrombolysis might represent an alternative or complementary approach. Here, we tested microbubble-mediated sonothrombolysis (mmSTL) in a thromboembolic stroke model for middle cerebral artery occlusion (MCAO) in rats.

Methods

Sixty-seven male Wistar rats underwent MCAO using an autologous full blood thrombus and were randomly assigned to 4 groups receiving rt-PA, mmSTL, a combination of both, or a placebo. Diagnostic workup included neurological examination, assessment of infarct size, and presence of intracerebral haemorrhage by magnetic resonance imaging (MRI) and presence of microbleedings in histological staining.

Results

Neurological examination revealed no differences between the treatment groups. In all treatment groups, there was a reduction in infarct size 24 hours after MCAO as compared to the placebo ($p \leq 0.05$), but there were no differences between the active treatment groups ($p > 0.05$) (Placebo 0.75 $cm^3 \pm 0.10$ cm^3; mmSTL 0.43 $cm^3 \pm 0.07$ cm^3; rt-PA 0.4 $cm^3 \pm 0.07$ cm^3; mmSTL + rt-PA 0.27 $cm^3 \pm 0.08$ cm^3). Histological staining displayed intracerebral microbleedings in all animals. The frequency of gross bleeding detected by MRI did not differ between the groups (Placebo 3; mmSTL 4; rt-PA 2; mmSTL + rt-PA 2; $p > 0.05$) and was not associated with worse performance in clinical testing ($p > 0.05$). There were no statistical differences in the mortality between the groups ($p > 0.05$).

Conclusions

Our study showed the efficacy and safety of microbubble-mediated sonothrombolysis with or without rt-PA in an embolic rat stroke model using a continuous full blood thrombus. Sonothrombolysis might be useful for patients who need to be transported to a thrombectomy centre or for those with distal vessel occlusion.

Key words: Embolic Stroke; Microbubbles; Rat; Sonothrombolysis; Ultrasound.

Introduction

Stroke is the second cause of death and the leading cause of disability in the world (1, 2). The majority of ischemic strokes is caused by cerebral embolism (3).

Achieving recanalization in a timely manner is a key goal in developing better stroke thrombolytic therapy. This is of importance in patients who are not eligible for recanalization therapy (intravenous recombinant tissue plasminogen activator [rt-PA] or thrombectomy) or to bridge time in patients with or without rt-PA treatment who need to be shipped from a remote hospital to a thrombectomy centre. In particular, patients with occlusion of smaller vessels need a reliable, effective, and safe therapy that can be performed in all stroke treating hospitals, as such a treatment is currently lacking.

Currently, the only approved thrombolytic treatment for acute ischemic stroke is rt-PA delivered intravenously within 4.5 hours of stroke onset (4). Less than 10% of all ischemic stroke patients are eligible for therapy, even though current research has suggested a higher rate when using elaborated imaging (perfusion imaging in computed tomography [CT] and magnetic resonance imaging [MRI]) for patient selection (5–8). However, only a small number of patients can be treated with rt-PA because there are several contraindications apart of the 4.5-hour “time window,” such as therapeutic anticoagulation, prior surgery, or active cancerous diseases. In Germany, 16.4% of patients admitted for ischemic stroke receive rt-PA treatment (9). However, rt-PA achieves successful recanalization in less than half of those treated (10). In patients with large vessel occlusion, recanalization can only be achieved in 10% of the patients using rt-PA (11). For these reasons, rt-PA treatment seems to have reached its limits. As recanalization is the most important factor for a satisfying patient outcome (12), there is dire need for a therapy that can achieve recanalization of an occluded vessel in a timely manner.

A complementary approach is catheter-based mechanical recanalization. This emerging treatment is gaining in importance and is associated with a satisfying patient outcome (13). Again, this approach is only possible in a minority of patients because it is currently only available in larger stroke centres and can only be used in proximal vessel occlusion. In Germany, 7.7% of patients admitted for ischemic stroke received this treatment in 2019 (9). The more distal vessels or smaller vessels cannot

be accessed by the thrombectomy catheters and can therefore not be recanalized by this approach, although the occlusion of those vessels can lead to detrimental effects for the patients, resulting in functional disability.

A promising approach to enhance recanalization with rt-PA is the use of ultrasound. The first clinical report of stroke sonothrombolysis indicated increased rates of recanalization in patients receiving continuous transcranial Doppler ultrasound (TCD) monitoring during rt-PA therapy (14). Several small clinical trials with perflutren-lipid and galactose-based microbubbles as enhancers of sonothrombolysis suggest that microbubbles may produce further improvements in the rates of recanalization (15–18). Despite the promise of this therapy, more than half of patients treated do not recanalize (19), and concerns have been raised regarding its safety with increased rates of haemorrhage in some studies (17, 20). The latest and largest trial in sonothrombolysis, the CLUTBUST-ER trial, demonstrated the safety of sonothrobolysis but failed to show beneficial effects on the clinical outcome (21). Therefore, there is still a great need for preclinical studies to better understand the efficacy and safety effects of this potential therapeutic strategy.

To test any thrombolytic therapy, a stroke model is required that uses a lifelike clot to block major cerebral arteries. Current thromboembolic models are highly variable, and it is likely that this variability is related to the choice of clot and its inherent stability with regards to spontaneous and thrombolytic-induced lysis (22).

In this article, we describe a method of embolic stroke using site-specific delivery of a whole blood thrombus to the origin of the middle cerebral artery (MCA). We aimed to investigate the effect of rt-PA therapy alone or in conjunction with ultrasound and a microbubble formulation (BR38; Bracco Suisse SA, Geneva, Switzerland) on recanalization rates in this model. BR38 is especially designed for usage in the therapy of acute intracranial artery occlusion and was described in detail by Schneider et al. (23).

We present the following article in accordance with the ARRIVE reporting checklist.

Methods

All experiments were performed in accordance with the German animal-protection legislation and were approved by the regional ethics committee of Regierungspraesidium Darmstadt (Az B 20/1120).

Sixty-seven male Wistar rats weighing 288 ± 24.81 g were used in the present study (Envigo RMS GmbH, In den Leppsteinswiesen 19, 64380 Roßdorf, Germany & Charles River, Sandhofer Weg 7, 97633 Sulzfeld, Germany).

The animals were anesthetized with 5% isoflurane inhalation for 2 minutes. Anaesthesia was maintained with 2% to 3% isoflurane inhalation at 0.5 L/min during surgery using a facial mask. For analgesia, the rats were treated with 0.05 mg/kg bodyweight burprenorphine (Buprenovet) 30 minutes prior to middle cerebral artery occlusion (MCAO) and in the evening after MCAO to ensure analgesia overnight. During surgery, 2% lidocaine was subcutaneously administered prior to cutting through the skin.

Body temperature was monitored and maintained at 36.5 °C to 37.0 °C. Dexpanthenol crème (Bepanthen, Bayer Vital) was used to prevent the eyes from drying out.

Thrombus preparation

The day before MCAO, the animals were anesthetized, and blood was taken from the tail vein. Ten 15-cm-long PE50 catheters were filled with blood, resulting in a total of 0.6 ml. After incubation of the catheters at 37 °C for 2 hours, a small part of the catheter was removed on both sides. Using a saline-filled syringe, the thrombi were flushed into a petri dish filled with saline. From the 10 resulting thrombi, a sufficiently long (4 cm) and uniformly thick and smooth thrombus was selected to be used for MCAO. The thrombus was then stored for 24 hours in a refrigerator at 4 °C.

MCAO

Rats were subjected to MCAO using a thromboembolic model as previously described (24). In short, the rat was anesthetized, and the right external carotid artery (ECA) and the right common carotid artery (CCA) were cauterized permanently, whereas a transient ligation of the internal carotid artery (ICA) was performed right

after the bifurcation. A PE10 catheter was introduced through a small arteriotomy in the ECA. The catheter was advanced over the CCA in the ICA beyond the pterygopalatine artery until a mild resistance was met at the origin of the MCA. The PE10 catheter was then connected to a second PE10 catheter that contained the aforementioned thrombus. The rat was then turned on the belly, and, after incision and mobilization of the scalp, a laser Doppler probe (Oxford Optronix probe with Oxyflow 2000 Microvascular Perfusion Monitor) was positioned between the bregma and the lambda suture. After ensuring a stable signal from the laser Doppler, the thrombus was then flushed from the catheter using 0.1-µl saline, resulting in a reduction of blood flow in the medial cerebral artery. A successful position of the thrombus resulted in a stable reduction of the blood flow to 50–90% from baseline. After waiting 40 minutes, treatment was started.

Treatment

For transcranial ultrasound application, the laser Doppler probe was removed, and the skin flaps of the scalp were held to the side using sutures. A 3-Mhz Doppler diagnostic ultrasound probe (Sonos 7500; Philips Ultrasound, USA; probe: diagnostic phased array probe with 80 elements) was placed above the skull, and the distance between the skull and ultrasonic probe was bridged with ultrasound gel (Transatlantic Handelsgesellschaft Stolpe & Co. mbH, Neu-Anspach, DE). Transcranial colour-coded duplex ultrasound (TCCD) was applied continuously for the duration of the treatment (B-mode, colour Doppler functions switched on, approximate size of the colour box: 4,9cm x 8,1cm). The Doppler beam was aligned to expose the entire brain incorporating the circle of Willis and the occluded MCA. The spectral Doppler sample volume (57 mm) was placed in the midbrain (pulse duration 2ms, maximum output [mechanical index of the Doppler of 1.7]). In rats without Doppler treatment (see below), the probe was placed, but the device remained deactivated.

The animals were randomized into 4 groups: rt-PA- & BR38-placebo without ultrasound (placebo), rt-PA-placebo & BR38 with ultrasound (US), rt-PA & BR-38 placebo without ultrasound (rt-PA), and rt-PA & BR38 with ultrasound (US + rt-PA). BR38 and rt-PA were administered via the tail vein. rt-PA (Actilyse, Boehringer Ingelheim, Ingelheim, Germany) was dosed at 10 mg/kg bodyweight (10% as bolus and 18% every 10 minutes). Four doses of 0.1-ml BR38 (10-µl BR 38 diluted in 90-µl saline; 4 x 10^8 bubbles/ml) were given at the start and every 15 minutes of treatment.

The corresponding amount of saline served as a placebo. Treatment duration was 60 minutes total.

Neurological examination and imaging

Neurological evaluation was performed prior to anaesthesia and 24 hours after induction of ischemia. We applied a neurological score with 10 different sensorimotor and coordinative items, as described by Nedelmann et al. (25). Furthermore, animals were placed on a rotarod that was continuously accelerated from 0 rounds per minute (rpm) to 30 rpm. The maximum speed that the animals tolerated without falling off the device was recorded (26). The rotarod scores before surgery and 24 hours after surgery were subtracted to display the deterioration.

The animals were subjected to MRI imaging 90 minutes and 24 hours after MCAO. MRI measurements were performed using a 7 Tesla MRI spectrometer (PharmaScan; Bruker, Billerica, Massachusetts, USA) equipped with a 760-mT/m gradient system using a 20-mm ^{1}H receive-only surface coil together with a 72-mm transmit-only volume resonator. For the MRI examination, the animals were fixed in a body restrainer with a tooth bar and a cone-shaped head. Respiratory rate was monitored by a pressure probe placed between the restrainer and the animal's thorax. Anaesthesia was maintained with isoflurane delivered through air at 0.6 L/min. The isoflurane concentration varied between 2.0% and 3.0% to keep the respiratory rate between 35 and 45/min. Temperature was monitored using a rectal probe and maintained at 37 °C by a thermostatically regulated water flow system during the entire imaging protocol.

Localizer images were acquired using a spin-echo sequence. RARE sequences (20 contiguous slices, 1 mm thickness, TR = 2500 ms, TE = 36.72 ms) were used to verify symmetric positioning and were repeated after correction of slice angulation, if necessary.

A Carr-Purcell-Meiboom-Gill spin-echo imaging sequence was used to map lesion and hemisphere volumes. Twelve contiguous coronal slices with a thickness of 1 mm (gap 0 mm) were acquired (FOV 35 × 35 mm, matrix 512 × 256, TR 3800 msec, 12 echoes: TE 18msec). Diffusion-weighted imaging was also used with a slice thickness of 1 mm (FOV 35 x 35 mm, matrix 128 x 128, TR 3000ms, TE 43 ms). Susceptibility-weighted sequences (SWI) with a slice thickness of 1 mm were used to

detect bleeding complications (FOV 35 x 35 mm, matrix 384 x 384, TR 720ms, TE 18 ms).

Infarct size and hemispheric volume were measured with planimetry in T2-weighted and diffusion-weighted sequences (Phillips Dicom Viewer 2.0). The SWI sequences were checked for the presence of intracerebral haemorrhage. Computer-aided planimetric assessments of ischemic lesion volumes and hemispheric volumes were performed by 2 blinded investigators experienced in experimental stroke MRI and sufficient training in planimetry (27). After optimal adjustment of contrast, the edges of the hemispheres were traced manually on each slice using neuroanatomic landmarks. The edges of the hyperintense ischemic lesions were traced manually in a similar fashion. The areas were then summed and multiplied by the slice thickness to calculate volumes. Lesion volumes were also expressed as a percent of the hemispheric volume.

Decapitation and histological staining

The animals were decapitated while under deep anaesthesia after completing the second MR imaging, and then the brains were quickly removed from the skull and inspected to detect side effects such as subarachnoid haemorrhage. The brains were fixed in a 4% formaldehyde solution (Roti® Histofix). After slicing and preparation, the slices were stained using haematoxylin and eosin. The slices were examined under the microscope and evaluated for microbleedings using a software (ZEN-Blue 2.6 by Zeiss).

Exclusion criteria

The animals were excluded in the case of severe dyspnoea and neurological impairment that made the ingestion of food and water impossible. If the MRI showed no infarction in the ipsilateral hemisphere, infarction in the contralateral hemisphere, or just a small infarction volume (< 0.1 cm³), the animals were excluded because of model failure.

Statistical analysis

Data are presented as mean ± standard deviation. All data was tested for normal distribution and variance homogeneity. Functional Assessment was tested using a T-Test. The difference in the groups in terms of bleeding frequencies and Mortality was

tested with an exact Fisher-Test. Group differences were tested using a mixed-model analysis (MMA). A p value ≤ 0.05 was considered statistically significant (SPSS v22, IBM Germany).

Results

Only 20 (29.9%) animals completed the experiment and were analysed per protocol. Twelve animals died during the surgery and the treatment procedure (17.9%). Another 10 animals died over the course of the night after the surgery (14.9%). Five animals (7.5%) had to be excluded due to dyspnoea or disabling neurological impairment. The MRI examination detected model failure in 20 (29.9%) animals.

For the distribution of the surviving animals, see **Table 1**.

Group	**Number of surviving animals**
Placebo	3
US	6
rt-PA	6
US + rt-PA	5

Table 1: Distribution of surviving animals in the different treatment groups

The physiological blood parameters, body temperature, and body weight of the remaining animals did not differ between the experimental groups and remained within the normal physiological range throughout the surgical and treatment procedure.

All surviving animals displayed clinical signs of MCA territory stroke 24 hours after the procedure. Clinical evaluation using the NeuroScore revealed no differences between groups (Placebo: 41.67 ± 4.07; US 32.5 ± 2.88; rt-PA 32.43 ± 3.12; US + rt-PA 36 ± 3.15; p > 0.05; MMA; **Figure 1**). Rotarod testing also showed no differences between the groups (Placebo: 2.67 rpm ± 3.09 rpm; US 6 rpm ± 2.19 rpm; rt-PA 4.67 rpm ± 2.19 rpm; US + rt-PA 5.6 rpm ± 2,4 rpm; p > 0.05; MMA; **Figure 2**).

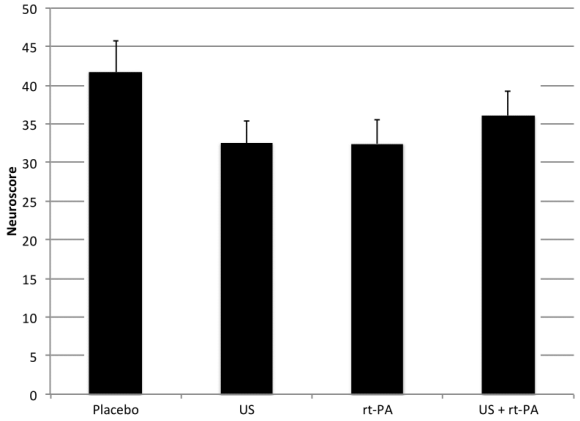

Figure 1: Mean value with standard deviation of the NeuroScore 24 hours after the thrombus induction. There is no significant difference between the 4 treatment groups (p > 0.05).

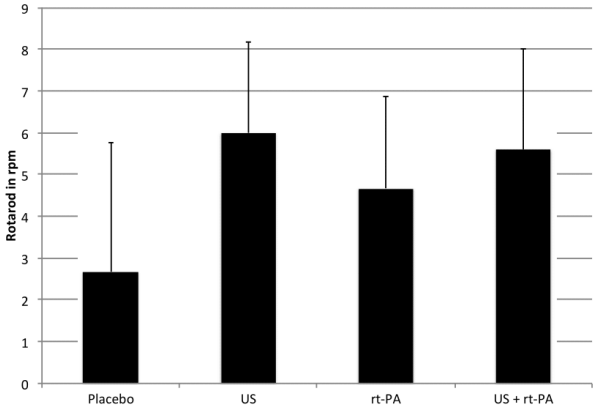

Figure 2: Mean value with standard deviation of the rotarod test in rounds per minute. The values show the difference between the first (preoperative) and second (postoperative) point of measurement. There is no significant difference between the 4 treatment groups ($p > 0.05$).

The absolute infarct size did not differ between the groups 90 minutes after surgery (Placebo 0.08 $cm^3 \pm 0.10$ cm^3; US 0.09 $cm^3 \pm 0.07$ cm^3; rt-PA 0.16 $cm^3 \pm 0.07$ cm^3; US + rt-PA 0.10 $cm^3 \pm 0.08$ cm^3; $p > 0.05$; MMA; **Figure 3**). There were also no differences when calculating the percentual infarct size of the affected hemisphere (Placebo 6.86% ± 9.09%; US 7.61% ± 6.43%; rt-PA 14.61% ± 6.43%; US + rt-PA 9.45% ± 7.04%; $p > 0.05$; MMA; **Figure 4**). **Figure 5** shows exemplary T2-weighted images of the different groups.

90min:

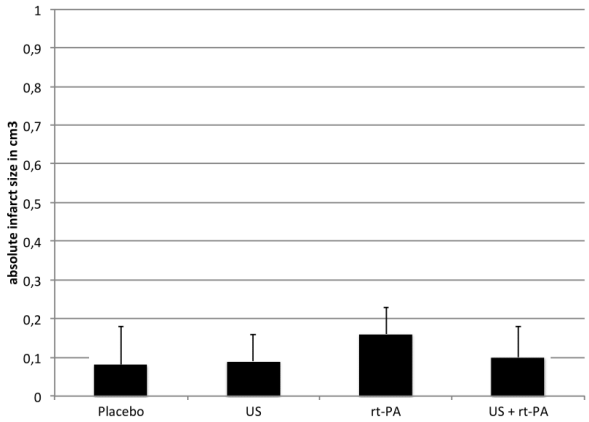

Figure 3: Mean value with standard deviation of the absolute infarct size in cm^3 90 minutes after thrombus induction. There is no significant difference between the 4 treatment groups ($p > 0.05$).

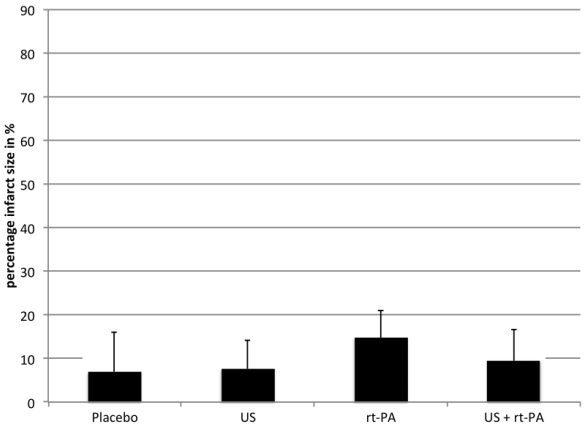

Figure 4: Mean value with standard deviation of the infarct size as percentage of ipsilateral hemispheric volume 90 minutes after thrombus induction. There is no significant difference between the 4 treatment groups ($p > 0.05$).

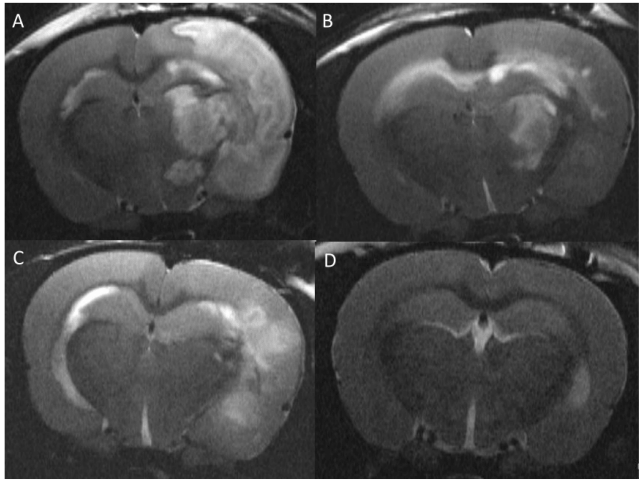

Figure 5: Examples of T2-weighted images of the infarct volume for the 4 different treatment groups. (A) Placebo (B) US (C) rt-PA (D) US + rt-PA.

Twenty-four hours after surgery, the placebo group had the largest ischemic volumes as compared to the treatment groups. Those differences were statistically significant. The lesional volume was smallest in the combined treatment group (US + rt-PA). However, there were no statistical differences when comparing the treatment groups, but all treatment groups were significantly smaller compared to Placebo (Placebo 0.75 $cm^3 \pm 0.10$ cm^3; US 0.43 $cm^3 \pm 0.07$ cm^3; rt-PA 0.4 $cm^3 \pm 0.07$ cm^3; US + rt-PA 0.27 $cm^3 \pm 0.08$ cm^3; $p > 0.05$; MMA; **Figure 6**). The same result was also found when calculating the infarct size as percentage of the ipsilateral hemispheric volume (Placebo 67.78% ± 9.09%; US 37.76% ± 6.43%; rt-PA 23.96% ± 7.04%; US + rt-PA 34.62% ± 6.43%; $p > 0.05$; MMA; **Figure 7**).

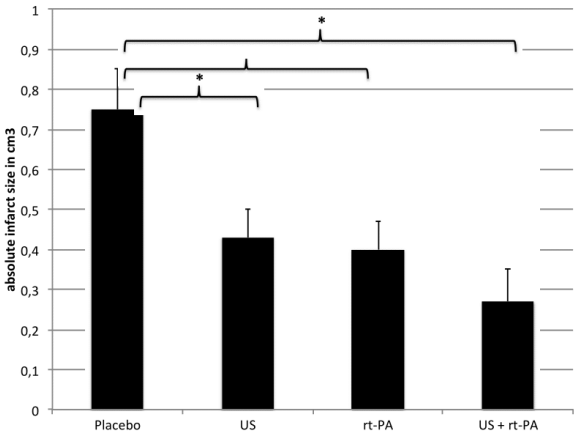

Figure 6: Mean value with standard deviation of the absolute infarct size in cm^3 24 hours after thrombus induction. There is a significant difference between Placebo and US ($p = 0.016$), Placebo and rt-PA ($p = 0.09$), and Placebo and US + rt-PA ($p = 0.001$). Asterisks are used to mark p-values below 0.05.

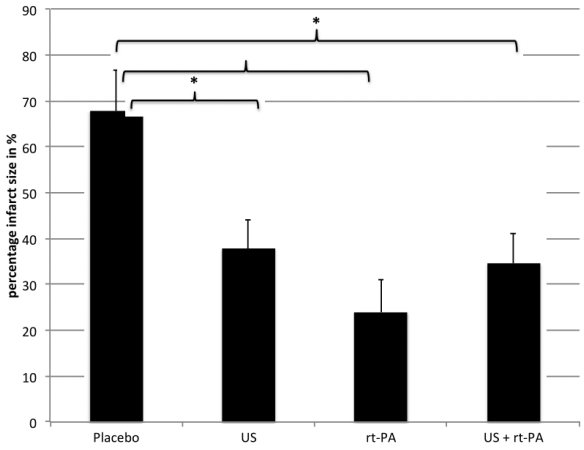

Figure 7: Mean value with standard deviation of the infarct size as percentage of ipsilateral hemispheric volume 24 hours after thrombus induction. There is a significant difference between Placebo and US (p = 0.012), Placebo and rt-PA (p = 0.006), and Placebo and US + rt-PA (p = 0.001). Asterisks are used to mark p-values below 0.05.

The absolute differences of ischemic volume 90 minutes and 24 hours after surgery were compared to visualize infarct growth. The largest growth was found in the placebo group (0.67 cm³; $p < 0.001$; MMA). There was also a statistically significant growth in the US group (0.34 cm³; $p < 0.001$; MMA) and the rt-PA group (0.24 cm³; $p < 0.007$; MMA). The US + rt-PA group showed the smallest growth that missed the significant level (0.17 cm³; $p < 0.062$; MMA).

Intracerebral bleeding was found using MRI imaging in 11 animals 24 hours after surgery. There were no differences between the groups (Placebo 3 [100%]); US 4 (67%); rt-PA 2 (33%); US + rt-PA 2 (40%); $p > 0.05$; Fisher-test). Clinical deficits and functional performance did not differ in animals with and without intracerebral bleeding in MRI imaging (NeuroScore: Bleeding 37.27 ± 8.17 No bleeding 31.25 ±

7.91; $p > 0.05$; t-test. Rotarod: Bleeding 3.27± 4.92 No bleeding 7.11 ± 6.79; $p > 0.05$ t-test). Histological staining revealed at least small intracerebral bleedings in all animals.

Of the 22 animals that died during surgery, 8 were in the placebo group (36.4%), 2 were in the US group (9.1%), 7 were in the rt-PA group (31.8%), and 5 were in the US + rt-PA group (22,7%). There were no statistical differences in the mortality between the groups ($p > 0.05$; Fisher-test).

Discussion

Our study showed a lower lesional volume in all animals with active therapy as compared to the placebo. There were no differences between the different treatment groups.

The safety evaluation of the different treatments using histological techniques revealed microbleedings in all animals, even in the placebo group. There were no statistical differences in the frequency of macroscopic intracerebral haemorrhage, detected by SWI sequences, or in mortality. Clinical performance of the animals with intracerebral haemorrhage did not differ from animals without intracerebral haemorrhage. As we saw no differences of intracerebral haemorrhage between the groups a delayed recanalization seems unlikely as a mechanism of action for STL. A delayed recanalization is associated with reperfusion injury and a higher rate of intracerebral haemorrhage (28).

90 minutes after thrombus induction, we saw no differences between the treatment groups. This might be mainly due to the circumstance, that T2-imaging, that reflects cerebral vasogenic oedema, needs time to show an ischemic lesion. 90 minutes after thrombus induction, there might remain some penumbral tissue that is still vital via collaterals that deteriorate over the course of time, resulting in infarct growth. The vasogenic oedema leads to secondary infarct growth due to compressive effects on cerebral vessels, but needs time to develop. It is responsible for up to 50% of the definite infarct volume (29).

Regarding the safety of microbubble-mediated sonothrombolysis (mmSTL), no existing experimental in vivo study revealed a higher rate of cerebral haemorrhage,

irrespective of stroke model, animal, microbubble use, or use of a concomitant thrombolytic agent (22). In most studies, mmSTL was classified as efficient. However, numerous primary endpoints were used such as lesional volume, presence of recanalization, declotting time, D-Dimer values, and clinical scores. Further methodological differences include the animal model (swine, rat, rabbit), clot preparation (autologous, human clots, red or white clots), route of microbubble administration (arterial or venous), ultrasound parameters, and use of rt-PA (22). This heterogeneity complicates the classification of our results.

Only 8 studies examined mmSTL in rats (30–37). In 6 of those studies, mmSTL was combined with rt-PA (30–34, 37).

Nedelmann et al. and Schleicher et al. used a filament model to assess microvascular impairment. Nedelmann et al. demonstrated less vascular impairment of the ipsilateral hemisphere after 60 minutes of transient MCAO when using mmSTL in combination with rt-PA as compared to the placebo, rt-PA alone, mmSTL alone, or the combination of rt-PA and sonothrombolysis without microbubbles. They also showed a lower infarct volume and less cerebral oedema but no differences in clinical outcome in mmSTL in combination with rt-PA as compared to rt-PA alone (33). Schleicher et al. examined rt-PA with mmSTL with 2 different microbubble types (Sonovue® and BR38®) and different microbubble dosages (full dose vs. 1/3 dose). They were able to demonstrate less vascular impairment of the ipsilateral hemisphere in all mmSTL-treated animals as compared to the controls or rt-PA alone. They also examined ischemic lesion volume histologically in controls, rt-PA only, and the combination of rt-PA and mmSTL (BR38®; full dosage). The combination of both therapies showed less ischemic volume as compared to the controls (31). Ren et al. and Lu et al. used fragmented thrombi to induce ischemia. When using a whole blood thrombus and Sonovue®, Ren et al. were able to show a faster recanalization when combining mmSTL with rt-PA after 10 minutes of treatment. However, rt-PA and a combination of mmSTL and rt-PA in halved dosages showed these results after 20 minutes of treatment. Twenty minutes after ischemia induction, mmSTL alone did not result in recanalization (32). Lu et al. used white and red thrombi in combination with a placebo, sonothrombolysis without microbubbles, mmSTL alone, rt-PA alone, or rt-PA combined with mmSTL. As a microbubble type, lipid shelled perfluoropropane microbubbles were used. mmSTL reduced the infarct

volume and showed a better clinical outcome irrespective of the used thrombi. mmSTL led to a lower infarct volume when using red thrombi compared to rt-PA, whereas there was a better clinical outcome in mmSTL and white thrombi compared to rt-PA. The combination of rt-PA with mmSTL led to a lower infarct volume when using red thrombi as compared to rt-PA alone. This effect was not reported when using white thrombi (34). Tomkins et al. used a continuous 3-cm-long white clot thrombus to achieve MCAO and BR38 as the microbubble type. The white clot proved to be resistant to the effects of mmSTL combined with rt-PA in this model (30).

Dixon et al. used a microfluidic device to produce microbubbles. MCAO was achieved by a continuous full blood thrombus. Microbubbles were directly administered into the ICA. They compared mmSTL with low-dose rt-PA to low-dose rt-PA alone, high-dose rt-PA alone, and controls. Ischemic volume was lower, and clinical outcome was better in high-dose rt-PA-treated animals as compared to the controls. There was no reduction in infarct volume in mmSTL with rt-PA as compared to the controls, but there was a better clinical outcome 24 hours after treatment (37).

Our study showed efficacy and safety of microbubble-mediated sonothrombolysis with or without t-PA in an embolic rat stroke model using a continuous full blood thrombus. It is difficult to compare our results to Dixon et al. They reported a clot volume of 16 µl, administered via a PE10 catheter. The diameter of the PE-10 catheter was not specified and varies among different manufacturers (37). When calculating the length using an inner diameter of 0.28 mm, and if all of this volume consisted of thrombus material, this would result in a ~26-cm-long thrombus administered to the rat. We were able to show an effect of mmSTL without use of rt-PA. Lu et al. and Re et al. demonstrated an effect of mmSTL without rt-PA in rats, but used a different clot model (32, 34). Brown et al. were also able to show an effect of mmSTL alone, but in rabbits (38). In vitro-studies have suggested, that moderate ultrasound intensities have a limited effect on thrombolysis without use of rt-PA (39). However, in the in vivo situation, intrinsic t-PA is present that might facilitate a thrombolytic effect.

Our experiment underlines the efficacy and safety of mmSTL in experimental in vivo stroke models. Nevertheless, in human stroke studies, a meta-analysis of sonothrombolysis with or without microbubble use showed no beneficial effects (16,

17, 40). Different contrast enhancers, such as Levovist®, Sonovue®, or microspheres, were used in the trials. In the largest trial, the CLOTBUST-ER trial, there was no use of a contrast enhancer (21). The use of mmSTL is partially limited by the administration of microbubbles, as they must be given either continually or with multiple boli over the course of the therapy. The relevance of sonothrombolysis in a clinical setting remains debatable, especially since the endovascular therapy studies demonstrated their large treatment effects. As Alexandrov et al. noted, new trials testing new non-endovascular approaches will be difficult to realize in the future, and it is questionable if new studies for sonothrombolysis will arise (41). Using a user independent head mount for insonation, the latest trial, the TRUST study, has not been recruiting since 2018 (source: clinicaltrials.gov/ct2/show/NCT03519737), but no results have yet been published. It remains questionable if sonothrombolysis has a role in cerebral large vessel occlusion, as those patients should undergo endovascular therapy. A scenario, in which a patient would not be eligible for this therapy, does not seem to be realistic. However, sonothrombolysis could be used during the transport from a primary stroke centre to a hospital for endovascular therapy. This setting was addressed in the TRUST trial. Patients with more distal vessel occlusion could also benefit from the effects of sonothrombolysis. Nevertheless, sonothrombolysis might share the fate of neuroprotection, as it might be effective in animal models but shows no effect on functional outcome of stroke patients. Therefore, sonothrombolysis will still play no role in clinical routine.

Our study is limited by the small number of animals completing the study protocol. However, we were able to show statistical differences regarding the efficacy of the treatment. There might be differences in the safety of the treatment that might not have been detected. As most animals died over the course of the night, we were unable to obtain direct information about the cause of death. Therefore, our study is also limited by the inability to differentiate if mortality resulted from the stroke model or as a side effect of the treatment. The short observation period after a stroke (24 hours) also limits our study because, at 24 hours after ischemia, the lesional size might not have reached its maximum. The outcome might not be stable, and late deficits could appear over the course of time (42). A longer observational period would not have been approved by our local animal ethics committee.

To improve the model, it should be possible to produce a thrombus that always has the same constitutions and comparable properties to humans. Thus, the scatter of infarct sizes would no longer exist and the non-responsive or dead animals can be eliminated in advance. The intraoperative mortality might be lower when using continuous pulse oximetry and orotracheal intubation. By this approach, lower dosages of isoflurane can be used which might lead to a lower rate of complications and the person performing the surgery can react faster to changes in SpO2 and the heart rate. By this approach, mortality and rate of model failure might be reduced.

Conclusions

Our study showed the efficacy and safety of microbubble-mediated sonothrombolysis with or without rt-PA in an embolic rat stroke model using a continuous full blood thrombus. Sonothrombolysis might be useful for patients who need to be transported to a thrombectomy centre or for those with distal vessel occlusion.

Acknowledgements

Funding: This research was supported by a research grant of the University Medical Center Giessen and Marburg (UKGM) [5/2016 GI] and by Bracco Suisse SA, Geneva, Switzerland.

Footnote

Reporting checklist: The authors have completed the ARRIVE reporting checklist.

Data sharing statement

The authors declare that all data were published in this manuscript.

Conflicts of interest: All authors have completed the ICMJE uniform disclosure form. TB reports grants from the University Medical Center Giessen and Marburg (UKGM), Germany; non-financial support from Bracco Suisse SA, Geneva, Switzerland, during the conduct of the study; non-financial support from Bayer Vital GmbH, outside the submitted work. The other authors have no conflicts of interest to declare.

No author serves currently as an Editorial Team member of this journal.

Ethical statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (NO.: Az B 20/1120) granted by the regional ethics committee of Regierungspraesidium Darmstadt, in compliance with German national guidelines for the care and use of animals.

Abbreviations

CCA	common carotid artery
CT	computed tomography
ECA	external carotid artery
ICA	internal carotid artery
MCAO	middle cerebral artery occlusion
MMA	mixed-model analysis
mmSTL	microbubble-mediated sonothrombolysis
MRI	magnetic resonance imaging
rpm	rounds per minute
rt-PA	recombinant tissue plasminogen activator
SWI	susceptibility-weighted imaging
TCD	transcranial Doppler ultrasound
TCCD	transcranial colour-coded duplex ultrasound
US	ultrasound

Supplementary material

None

References

1. Benjamin E, Blaha M, Chiuve S et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation 2017;135:e146-e603. doi:10.1161/CIR.0000000000000485.
2. Mathers C, Boerma T, Ma Fat D. Global and regional causes of death. Br Med Bull 2009;92:7–32. doi:10.1093/bmb/ldp028.
3. Ntaios G, Hart R. Embolic Stroke. Circulation 2017;136:2403–5. doi:10.1161/CIRCULATIONAHA.117.030509.
4. Hacke W, Kaste M, Bluhmki E et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008;359:1317–29. doi:10.1056/NEJMoa0804656.
5. Goyal M, Demchuk A, Menon B et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med 2015;372:1019–30. doi:10.1056/NEJMoa1414905.
6. Ma H, Campbell B, Parsons M et al. Thrombolysis Guided by Perfusion Imaging up to 9 Hours after Onset of Stroke. N Engl J Med 2019;380:1795–803. doi:10.1056/NEJMoa1813046.
7. Nogueira R, Jadhav A, Haussen D et al. Thrombectomy 6 to 24 Hours after Stroke with a Mismatch between Deficit and Infarct. N Engl J Med 2018;378:11–21. doi:10.1056/NEJMoa1706442.
8. Thomalla G, Simonsen C, Boutitie F et al. MRI-Guided Thrombolysis for Stroke with Unknown Time of Onset. N Engl J Med 2018;379:611–22. doi:10.1056/NEJMoa1804355.
9. Richter D, Eyding J, Weber R et al. Analysis of Nationwide Stroke Patient Care in Times of COVID-19 Pandemic in Germany. Stroke 2020:STROKEAHA120033160. doi:10.1161/STROKEAHA.120.033160.
10. Rha J-H, Saver J. The impact of recanalization on ischemic stroke outcome: a meta-analysis. Stroke 2007;38:967–73. doi:10.1161/01.STR.0000258112.14918.24.
11. Tsivgoulis G, Katsanos A, Schellinger P et al. Successful Reperfusion With Intravenous Thrombolysis Preceding Mechanical Thrombectomy in Large-Vessel Occlusions. Stroke 2018;49:232–5. doi:10.1161/STROKEAHA.117.019261.

12. Rabinstein A. Treatment of Acute Ischemic Stroke. Continuum (Minneap Minn) 2017;23:62–81. doi:10.1212/CON.0000000000000420.
13. Balami J, Sutherland B, Edmunds L et al. A systematic review and meta-analysis of randomized controlled trials of endovascular thrombectomy compared with best medical treatment for acute ischemic stroke. Int J Stroke 2015;10:1168–78. doi:10.1111/ijs.12618.
14. Alexandrov A, Demchuk A, Felberg R et al. High rate of complete recanalization and dramatic clinical recovery during tPA infusion when continuously monitored with 2-MHz transcranial doppler monitoring. Stroke 2000;31:610–4. doi:10.1161/01.STR.31.3.610.
15. Alexandrov A, Mikulik R, Ribo M et al. A pilot randomized clinical safety study of sonothrombolysis augmentation with ultrasound-activated perflutren-lipid microspheres for acute ischemic stroke. Stroke 2008;39:1464–9. doi:10.1161/STROKEAHA.107.505727.
16. Molina C, Barreto A, Tsivgoulis G et al. Transcranial ultrasound in clinical sonothrombolysis (TUCSON) trial. Ann Neurol 2009;66:28–38. doi:10.1002/ana.21723.
17. Molina C, Ribo M, Rubiera M et al. Microbubble administration accelerates clot lysis during continuous 2-MHz ultrasound monitoring in stroke patients treated with intravenous tissue plasminogen activator. Stroke 2006;37:425–9. doi:10.1161/01.STR.0000199064.94588.39.
18. Viguier A, Petit R, Rigal M et al. Continuous monitoring of middle cerebral artery recanalization with transcranial color-coded sonography and Levovist. J Thromb Thrombolysis 2005;19:55–9. doi:10.1007/s11239-005-0940-6.
19. Alexandrov A, Molina C, Grotta J et al. Ultrasound-enhanced systemic thrombolysis for acute ischemic stroke. N Engl J Med 2004;351:2170–8. doi:10.1056/NEJMoa041175.
20. Daffertshofer M, Gass A, Ringleb P et al. Transcranial low-frequency ultrasound-mediated thrombolysis in brain ischemia: increased risk of hemorrhage with combined ultrasound and tissue plasminogen activator: results of a phase II clinical trial. Stroke 2005;36:1441–6. doi:10.1161/01.STR.0000170707.86793.1a.
21. Alexandrov A, Köhrmann M, Soinne L et al. Safety and efficacy of sonothrombolysis for acute ischaemic stroke: a multicentre, double-blind, phase

3, randomised controlled trial. Lancet Neurol 2019;18:338–47. doi:10.1016/S1474-4422(19)30026-2.
22. Auboire L, Sennoga C, Hyvelin J-M et al. Microbubbles combined with ultrasound therapy in ischemic stroke: A systematic review of in-vivo preclinical studies. PLoS ONE 2018;13:e0191788. doi:10.1371/journal.pone.0191788.
23. Schneider M, Anantharam B, Arditi M et al. BR38, a new ultrasound blood pool agent. Invest Radiol 2011;46:486–94. doi:10.1097/RLI.0b013e318217b821.
24. Zhang R, Chopp M, Zhang Z et al. A rat model of focal embolic cerebral ischemia. Brain Res 1997;766:83–92. doi:10.1016/s0006-8993(97)00580-5.
25. Nedelmann M, Wilhelm-Schwenkmezger T, Alessandri B et al. Cerebral embolic ischemia in rats: correlation of stroke severity and functional deficit as important outcome parameter. Brain Res 2007;1130:188–96. doi:10.1016/j.brainres.2006.10.087).
26. Hamm R, Pike B, O'Dell D et al. The rotarod test: an evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. J Neurotrauma 1994;11:187–96. doi:10.1089/neu.1994.11.187.
27. Braun T, Pukropski J, Yeniguen M et al. Inter- and intra-rater reliability of computer-assisted planimetry in experimental stroke research. J Neurosci Methods 2019;312:12–5. doi:10.1016/j.jneumeth.2018.11.012.
28. Molina C, Montaner J, Abilleira S et al. Timing of spontaneous recanalization and risk of hemorrhagic transformation in acute cardioembolic stroke. Stroke 2001;32:1079–84. doi:10.1161/01.str.32.5.1079.
29. Walberer M, Ritschel N, Nedelmann M et al. Aggravation of infarct formation by brain swelling in a large territorial stroke: a target for neuroprotection? J Neurosurg 2008;109:287–93. doi:10.3171/JNS/2008/109/8/0287.
30. Tomkins A, Schleicher N, Murtha L et al. Platelet rich clots are resistant to lysis by thrombolytic therapy in a rat model of embolic stroke. Exp Transl Stroke Med 2015;7:2. doi:10.1186/s13231-014-0014-y.
31. Schleicher N, Tomkins A, Kampschulte M et al. Sonothrombolysis with BR38 Microbubbles Improves Microvascular Patency in a Rat Model of Stroke. PLoS ONE 2016;11:e0152898. doi:10.1371/journal.pone.0152898.
32. Ren X, Wang Y, Wang Y et al. Thrombolytic therapy with rt-PA and transcranial color Doppler ultrasound (TCCS) combined with microbubbles for embolic thrombus. Thromb Res 2015;136:1027–32. doi:10.1016/j.thromres.2015.08.027.

33. Nedelmann M, Ritschel N, Doenges S et al. Combined contrast-enhanced ultrasound and rt-PA treatment is safe and improves impaired microcirculation after reperfusion of middle cerebral artery occlusion. J Cereb Blood Flow Metab 2010;30:1712–20. doi:10.1038/jcbfm.2010.82.
34. Lu Y, Wang J, Huang R et al. Microbubble-Mediated Sonothrombolysis Improves Outcome After Thrombotic Microembolism-Induced Acute Ischemic Stroke. Stroke 2016;47:1344–53. doi:10.1161/STROKEAHA.115.012056.
35. Alonso A, Dempfle C-E, Della Martina A et al. In vivo clot lysis of human thrombus with intravenous abciximab immunobubbles and ultrasound. Thromb Res 2009;124:70–4. doi:10.1016/j.thromres.2008.11.019.
36. Moumouh A, Barentin L, Tranquart F et al. Fibrinolytic effects of transparietal ultrasound associated with intravenous infusion of an ultrasound contrast agent: study of a rat model of acute cerebral stroke. Ultrasound Med Biol 2010;36:51–7. doi:10.1016/j.ultrasmedbio.2009.06.1103.
37. Dixon A, Li J, Rickel J-M et al. Efficacy of Sonothrombolysis Using Microbubbles Produced by a Catheter-Based Microfluidic Device in a Rat Model of Ischemic Stroke. Ann Biomed Eng 2019;47:1012–22. doi:10.1007/s10439-019-02209-0.
38. Brown A, Flores R, Hamilton E et al. Microbubbles improve sonothrombolysis in vitro and decrease hemorrhage in vivo in a rabbit stroke model. Invest Radiol 2011;46:202–7. doi:10.1097/RLI.0b013e318200757a.
39. Bader K, Gruber M, Holland C. Shaken and stirred: mechanisms of ultrasound-enhanced thrombolysis. Ultrasound Med Biol 2015;41:187–96. doi:10.1016/j.ultrasmedbio.2014.08.018.
40. Nacu A, Kvistad C, Naess H et al. NOR-SASS (Norwegian Sonothrombolysis in Acute Stroke Study): Randomized Controlled Contrast-Enhanced Sonothrombolysis in an Unselected Acute Ischemic Stroke Population. Stroke 2017;48:335–41. doi:10.1161/STROKEAHA.116.014644.
41. Alexandrov A, Tsivgoulis G, Köhrmann M et al. Endovascular equipoise shift in a phase III randomized clinical trial of sonothrombolysis for acute ischemic stroke. Ther Adv Neurol Disord 2019;12:1756286419860652. doi:10.1177/1756286419860652.
42. Kahle M, Bix G. Successfully Climbing the "STAIRs": Surmounting Failed Translation of Experimental Ischemic Stroke Treatments. Stroke Res Treat 2012;2012:374098. doi:10.1155/2012/374098.

Tables

Table 1:

Group	Number of surviving animals
Placebo	3
US	6
rt-PA	6
US + rt-PA	5

Table 1 - Distribution of surviving animals in the different treatment groups

Figures

Figure 1 - Mean value with standard deviation of the NeuroScore 24 hours after the thrombus induction. There is no significant difference between the 4 treatment groups ($p > 0.05$).

Figure 2 – Mean value with standard deviation of the rotarod test in rounds per minute. The values show the difference between the first (preoperative) and second (postoperative) point of measurement. There is no significant difference between the 4 treatment groups ($p > 0.05$).

Figure 3 – Mean value with standard deviation of the absolute infarct size in cm^3 90 minutes after thrombus induction. There is no significant difference between the 4 treatment groups ($p > 0.05$).

Figure 4 – Mean value with standard deviation of the percentage infarct size in cm^3 90 minutes after thrombus induction. There is no significant difference between the 4 treatment groups ($p > 0.05$).

Figure 5 – Examples of T2-weighted images of the infarct volume for the 4 different treatment groups. (A) Placebo (B) US (C) rt-PA (D) US + rt-PA.

Figure 6 – Mean value with standard deviation of the absolute infarct size in cm^3 24 hours after thrombus induction. There is a significant difference between Placebo and US ($p = 0.016$), Placebo and rt-PA ($p = 0.09$), and Placebo and US + rt-PA ($p = 0.001$).

Figure 7 – Mean value with standard deviation of the percentage infarct size in cm^3 24 hours after thrombus induction. There is a significant difference between Placebo and US ($p = 0.012$), Placebo and rt-PA ($p = 0.006$), and Placebo and US + rt-PA ($p = 0.001$).

[ATM] Annals of Translational Medicine - Acceptance of Manu... - Br... https://owa.med.uni-giessen.de/owa/#viewmodel=ReadMessageItem&...

[ATM] Annals of Translational Medicine - Acceptance of Manuscript ATM-21-75

Darren Yu <editor@atmjournal.org>

Mi 14.04.2021 11:21

An:Braun, Tobias <Tobias.Braun@neuro.med.uni-giessen.de>;

Cc:Laura Suenner <laura.suenner@t-online.de>; Maaike Hachenberger <maaike.hachenberger@neuro.med.uni-giessen.de>; Clemens Mueller <c.mueller@kerckhoff-klinik.de>; Astrid Wietelmann <astrid.wietelmann@mpi-bn.mpg.de>; Pons, Joern <Joern.Pons@informatik.med.uni-giessen.de>; Kaps, Manfred <manfred.kaps@neuro.med.uni-giessen.de>; Gerriets, Tibo <tibo.gerriets@neuro.med.uni-giessen.de>; Tschernatsch, Marlene <Marlene.Tschernatsch@neuro.med.uni-giessen.de>; Joachim Roth <Joachim.Roth@vetmed.uni-giessen.de>; Yeniguen, Mesut <Mesut.Yeniguen@neuro.med.uni-giessen.de>;

1 Anlage

TH2021040347-ATM-21-75-Bill.pdf;

Bill Number: TH2021040347
Manuscript ID: ATM-21-75
Title: Microbubble-Mediated Sonothrombolysis with BR38 of a Venous Full Blood Thrombus in a Rat Embolic Stroke Model

Dear Dr. Braun,

The Editorial Office of Annals of Translational Medicine is pleased to inform you that your above manuscript has been accepted for publication in Annals of Translational Medicine. Congratulations!

1. Authors Column
We are glad to let you know that ATM editorial office has launched a column "Authors" to appreciate the efforts and supports from our authors and highlight their works. All your articles published in ATM will be listed in your profile under this column. If you are interested, please provide us with your recent photo in JPG and a brief biography in DOC format. Here is an example for your reference: http://atm.amegroups.com/user/view/98269. Your profile will be posted online after the article is published.

2. Publication process
In accordance with our early agreement regarding the article processing fee, you will be charged the article processing fee (USD 1390) prior to the publication of the article. Your article will be proceeded with copy-editing and type-setting soon after the payment has been received.

To ensure timely publication of your manuscript, please send the Payment Proof (bank slip or screenshot) to editor@atmjournal.org and cc to tanch@amegroups.com within 2 weeks. Please note that your manuscript may be rejected automatically if we do not hear from you within 2 weeks. Change of author information (except for grammatical error) is not allowed after the manuscript is accepted.

Attached is the bill for your reference. Please follow the instructions inside the bill and mark the Bill Number/Manuscript ID with all payments. Payments submitted without a Bill Number will not be applied to the outstanding bill.

Thank you for your fine contribution. On behalf of the editors of Annals of Translational Medicine, we look forward to your continued contributions to the journal.

If you fail to download or open the attachment, please click the link below to download the file:
http://atm.amegroups.com/user/download?name=pdfN02rAW&type=application/pdf&original_name=TH2021040347-ATM-21-75-Bill.pdf.

Yours sincerely,

_____
Editorial Office
Annals of Translational Medicine
Web: http://atm.amegroups.com

_____
Editorial Office
Annals of Translational Medicine
Web: http://atm.amegroups.com

2 von 2 17.05.2021, 21:24

5.4. Validation of the German Version of Functional Oral Intake Scale (FOIS-G) for Flexible Endoscopic Evaluation of Swallowing (FEES)[210]

Samra Hamzic*, **Tobias Braun***, Martin Jünemann, Marius Butz, Robert Voswinckel, Michael Belly, Oliver Vogelbusch, Susanne Weber, Hasan Khilan, Manfred Kaps, Tibo Gerriets

*contributed equally

Die “Functional Oral Intake Scale” (FOIS) ist die am häufigsten verwendete Skala, um die funktionelle Ernährungsweise von Dysphagiepatienten zu beschreiben. Die FOIS wurde anhand videofluoroskopischer Untersuchungen des Schluckes validiert. Da bis dahin keine deutschsprachige Version für die FEES-Untersuchung validiert wurde, erfolgte diese Studie. Das Ziel war es, eine interkulturelle Validierung einer deutschen Version der FOIS (FOIS-G) für die FEES durchzuführen.

Die Übersetzung der originalen FOIS-Skala erfolgte gemäß den TRAPD-Richtlinien für die Übersetzung methodischer Arbeiten. Hierbei steht TRAPD für Translation, Review, Adjudication, Pretesting, Documentation (Übersetzung, Überprüfung, Entscheidung, Vortestung, Dokumentation).

Für den Validierungsprozess analysierten sechs erfahrene Logopäden retrospektiv die Patientenakten von 93 Schlaganfallpatienten. Die Einschlusskriterien umfassten die Untersuchung durch einen Logopäden innerhalb von 24 Stunden nach Aufnahme und die Durchführung einer FEES-Untersuchung bis 72 Stunden nach Aufnahme. Die Validität wurde berechnet durch den Vergleich mit der modifizierten Rankin Skala (mRS), dem Barthel Index (BI), der Penetrations-Aspirations-Skala (PAS) und einem 70ml-Wasser-Schlucktest. Die Spearmann-Rangkorrelation der gepaarten Bewerter lag zwischen rs = 0,96 und rs = 0,99. Die prozentuale Übereinstimmung lag zwischen 81 und 94%. Die Gesamtübereinstimmung wurde mittels Fleiss‘ Kappa berechtnet und lag bei 0,83 (s.e. 0,02). Es lag eine signifikante Korrelation zwischen BI und mRS mit dem FOIS-G-Wert (BI: rs = 0,301, p = 0,003; mRS: rs = – 0.366, p < 0.001), zwischen PAS und dem FOIS-G-Wert (rs = − 0,758, p < 0,001) und zwischen dem Ergebnis des 70ml-Wasser-Schlucktest und dem FOIS-G-Wert vor (rs = 0,470, p < 0,001).

Zusammenfassend konnte die FOIS-G als valides Messinstrument für die funktionelle Ernährungsweise mit Nahrung und Flüssigkeiten bei Dysphagiepatienten bestätigt werden.

Dysphagia
https://doi.org/10.1007/s00455-020-10114-1

ORIGINAL ARTICLE

Validation of the German Version of Functional Oral Intake Scale (FOIS-G) for Flexible Endoscopic Evaluation of Swallowing (FEES)

Samra Hamzic[1] · Tobias Braun[1] · Martin Juenemann[1] · Marius Butz[1] · Robert Voswinckel[2] · Michael Belly[2] · Oliver Vogelbusch[1] · Susanne Weber[2] · Hasan Khilan[2] · Manfred Kaps[1] · Tibo Gerriets[1,2]

Received: 16 April 2019 / Accepted: 15 April 2020

Abstract
The Functional Oral Intake Scale (FOIS) is the most frequently used scale for the evaluation of functional oral intake by dysphagia patients. FOIS was validated using data from Videofluoroscopic Swallowing Study (VFSS). Until now, a validated German version of FOIS for Flexible Endoscopic Evaluation of Swallowing (FEES) is lacking. The aim of this study was a cross-cultural validation of the German version of FOIS (FOIS-G) for FEES. The translation of the original FOIS was carried out according to the Translation, Review, Adjudication, Pretesting, Documentation (TRAPD) translation methodology. For the validation process, six experienced language therapists (SLT) retrospectively analyzed charts of 93 stroke patients. Inclusion criteria were comprised of stroke, clinical examination by an SLT within 24 h of admission, and FEES within 72 h of admission. The validity was calculated by comparison with Modified Rankin Scale (MRS), Barthel Index (BI), the Penetration-Aspiration-Scale (PAS), and a water swallow test. Spearman rank correlation of all paired raters ranged from $r_s = 0.96$ to $r_s = 0.99$, and percentage agreement ranged from 81 to 94%. The overall agreement between all raters was calculated by Fleiss kappa (0.83) (s.e. 0.02). There is a significant correlation between the BI and the MRS with the FOIS-G ($r_s = 0.301$, $p = 0.003$ for BI; $r_s = -0.366$, $p < 0.001$ for MRS), between the PAS and the FOIS-G ($r_s = -0.758$, $p < 0.001$), as well as between the 70 ml-water-test and the FOIS-G ($r_s = 0.470$, $p < 0.001$). FOIS-G is a valid instrument for the evaluation of the functional oral intake of food and liquids in dysphagia patients.

Keywords Dysphagia · Deglutition · Deglutition disorders · FOIS · Cross-cultural adaptation · FEES · German version · Validation

Introduction

Neurogenic dysphagia comprises of a disordered intake of fluids and food due to neurologic diseases. It causes restrictions in patients' oral ability to intake and process secretions, food, and fluids. Dysphagia may be a cause of malnutrition, dehydration, and aspiration pneumonia and can entail a prolonged length of hospital stay. As a consequence, patients may encounter long-term artificial nutrition, invasive ventilation via tracheotomy tubes, reduced quality of life, and, lastly, death [1–4].

Dysphagia is a common consequence of a stroke. Its incidence among stroke survivors shows a high degree of variability ranging from 19 to 81% when imaging methods for dysphagia like Videofluoroscopic Swallowing Study (VFSS) or Flexible Endoscopic Evaluation of Swallowing (FEES) are implemented [1, 5–8]. Six months after stroke, up to 50% of patients still suffer dysphagia [9, 10].

Early detection of dysphagia is beneficial for the overall outcome by reducing the risks of mortality and of secondary complications such as aspiration pneumonia, dehydration, and malnutrition as well as the length of hospitalization and the overall costs of treatment [11].

An adequate care of dysphagia patients includes the application of validated clinical and instrumental diagnostic

Samra Hamzic and Tobias Braun have contributed equally to this work.

✉ Samra Hamzic
samra.hamzic@neuro.med.uni-giessen.de

[1] Department of Neurology, University Hospital Giessen and Marburg GmbH, Klinikstrasse 33, 35392 Giessen, Hesse, Germany

[2] Buergerhospital Friedberg (Hesse), Stroke Unit, Ockstaedter Str. 3-5, 61169 Friedberg, Hesse, Germany

Published online: 27 April 2020

methods and scales. An imaging method, FEES, has become the gold standard in Germany and has been implemented in more than 70% of stroke units [12]. The scores most frequently used for objective evaluation of dysphagia severity are the Penetration-Aspiration-Scale (PAS) [13], the Secretion Severity Rating Scale (SSRS) [14] and the FOIS scale [15]. These scores allow for monitoring of swallowing ability and security over time.

The functional oral intake scale (FOIS) was developed in 2005 as a tool with very good reliability, validity, and sensitivity to change to objectively determine and monitor the range of oral intake of patients with neurogenic dysphagia [15]. It is an ordinal scale with seven tiers that assesses the oral intake of food and liquids. Different ranges of non-oral feeding are subsumed in levels 1–3, whereas different ranges of oral feeding are included in levels 4–7. It has been the most commonly used scale for the rating of the range of oral intake by patients suffering from dysphagia and is used both in clinical and in research settings [16, 17] as well as in various patient populations (patients with amyotrophic lateral sclerosis, head and neck cancer, Parkinson's disease, and pediatric patients) [18–21].

Functional rating scales have been applied as assessment protocols, tools for evaluation of patient outcomes and for detection of changes in swallowing over time [22]. Furthermore, they can be used to monitor the adequacy and effectivity of training and rehabilitation methods. Compared to Functional Outcome Swallowing Scale (FOSS) [23], the Food Intake Level Scale (FILS) [24] and the Dysphagia Outcome and Severity Scale (DOSS) [25], each lacking either reliability, validity, or sensitivity to change over time, FOIS is an impairment-specific scale with precisely defined differences between easily understood scale levels and an excellent psychometric quality. In our experience and as confirmed in various studies [16–21], FOIS has shown to be an excellent and very practical tool for assessing functional oral intake in dysphagic patients and for monitoring rehabilitation achievements over time. We are committed to the use of best validated procedures and scores for our patients to monitor the range of oral intake and the efficacy of both dysphagia and nutritional treatment. The lack of a uniform worldwide approach and guidelines for patient-oriented, time- and cost-effective dysphagia management is a well-known fact [26, 27]. The implementation of validated scales like FOIS in several languages is an important step in this direction and paves the way to maximize comparability of international research. The aim of this study is to satisfy these demands for the German language and to conduct a cross-cultural validation of the German version of the FOIS scale (FOIS-G).

For the translation process, we implemented the TRAPD-procedures (Translation, Review, Adjudication, Pretesting and Documentation) and a committee-based approach to translation process, which does not include the back translation methodology [28].

The TRAPD-method as a five-step team-based approach suggests parallel translation of the source text in cooperation between three different sets of persons: translators, reviewers, and adjudicators [29]. All members own a mixture of skills and expertise allowing for an optimal decision on the best version. The team has a profound knowledge of the study issue, the measures to be translated, and the underlying research topic. Finally, all team members need to possess a high level of linguistic and cultural knowledge in order to establish an adequate version in the target language [29–34]. According to the TRAPD-method, more than one translator is needed for the translation from the source into the target language. At least one person, who is experienced in the principles of questionnaires and surveys design, linguistics, and translations, is also included in the reviewing process. The adjudicator is specialized in the research topic, having knowledge of both the target and the original language and is in charge of all final decisions concerning the final translation version and can take the role both of reviewer and adjudicator ("reviewer cum adjudicator") [29].

Materials and Methods

Translation Process According to TRAPD-Methodology

Phase 1: Translation

For the forward translation from English into German the parallel translation method was selected: two neurologists and a speech and language therapist (SLT), who are active in dysphagia research and have a proficient and fluent command of written and spoken English, produced independently parallel translation drafts.

Phase 2: Review

The review of the forward translation drafts was assigned to the translators and two reviewers (first author of this article being one of them). The goal of the review step was to identify discrepancies and special difficulties between the original scale and the three parallel translations and decide on a preliminary version of FOIS-G.

Phase 3: Adjudication

In a joint expert panel, all persons included in the forward translation and the review process discussed the final version of FOIS-G to be adopted. For this expert panel, the author of this article was in charge as reviewer cum adjudicator

for the validation process: Modified Rankin Scale (MRS), the Modified Barthel Index (MBI), and Mann Assessment of Swallowing Ability (MASA). Finally, all patients underwent a VFSS within 72 h of admission in which the severity of dysphagia and aspiration presence and severity was assessed.

The MRS, as a 7-tiered scale, is a functional outcome measure for stroke patients measuring the level of disability, whereas MBI scores the dependence of stroke survivors in activities of daily living after stroke. In clinical trials, MRS and MBI are frequently implemented as primary outcome measures.

MASA is a bedside screening tool to detect swallowing disorders and aspiration in acute stroke patients showing significant sensitivity and specificity [35–37]. However, the clinical screening tool for dysphagia most frequently used in German stroke units is the water-test according to Daniels (further referring to as 70 ml-water-test), which shows a 93% rate of sensitivity and a 67% rate of specificity in detecting aspiration risk in acute stroke patients [38].

In contrast to VFSS, which is the gold standard of imaging diagnostics in the United States, FEES is the method of choice in Germany [12].

For our validation study, we used following outcome measures, which are commonly assessed in German stroke units: the MRS, the standard Barthel Index (BI), the 70 ml-water-test and the PAS score for FEES (Fig. 3, Table 2).

FEES Methodology

The clinician performing FEES is an experienced SLT and dysphagia therapist and holder of the FEES Instructor Certificate of the German Society of Neurology and the European Society for Swallowing Disorders with more than ten years of experience in FEES in conducting evaluation and research. The FEES examination is carried out in three sections: (1) examination of anatomical structures and secretion rating, (2) swallow examination, and (3) symptoms evaluation. Validated scales are used for the evaluation of swallowing: The Secretion Severity Rating Scale (SSRS) [14], the Penetration-Aspiration-Scale (PAS) [13], and the Yale Pharyngeal Residue Severity Rating Scale for Valleculae and Piriform sinus (Yale Scale V/PS) [39]. Exactly defined amounts of liquid (1 teaspoon = 3 ml; 1 sip = 10 ml), pureed (1 teaspoon = 4 ml), and solid boluses (5 g) are administered each three times to the patients. Following cutoff values of the scales for saliva (SSRS = 3, PAS ≥ 7, Yale Scale Valleculae /Piriform sinus = V), liquid ((PAS ≥ 7), pureed (PAS ≥ 7), and solid boluses (PAS ≥ 7) determine when to abort the swallow examination. Finally, the FOIS scale and oral diet are recorded after the evaluation of swallowing capacity on the basis of perceived symptoms and determined scale scores.

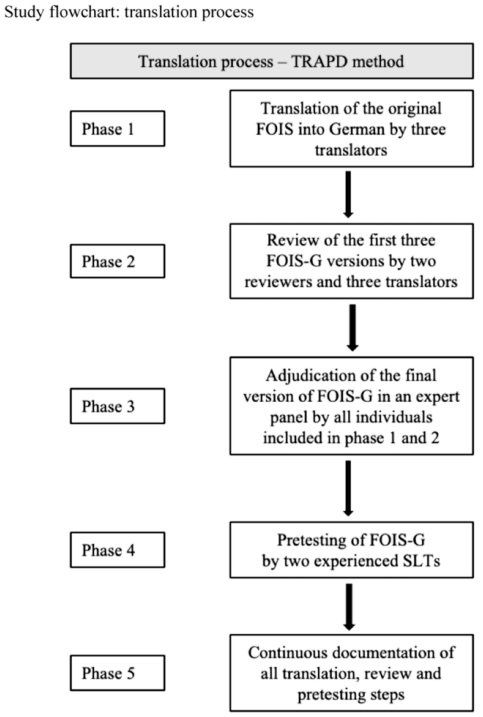

Fig. 1 Study flowchart: translation process

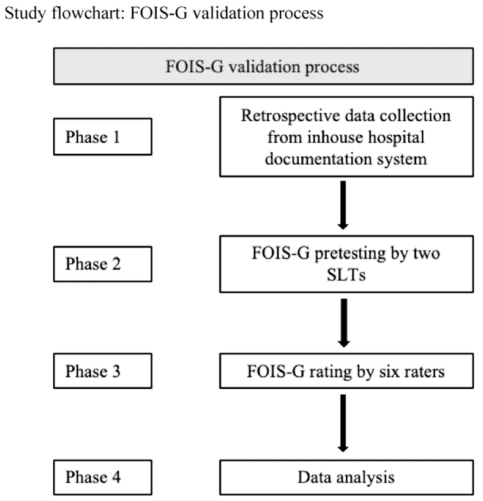

Fig. 2 Study flowchart: FOIS-G validation process

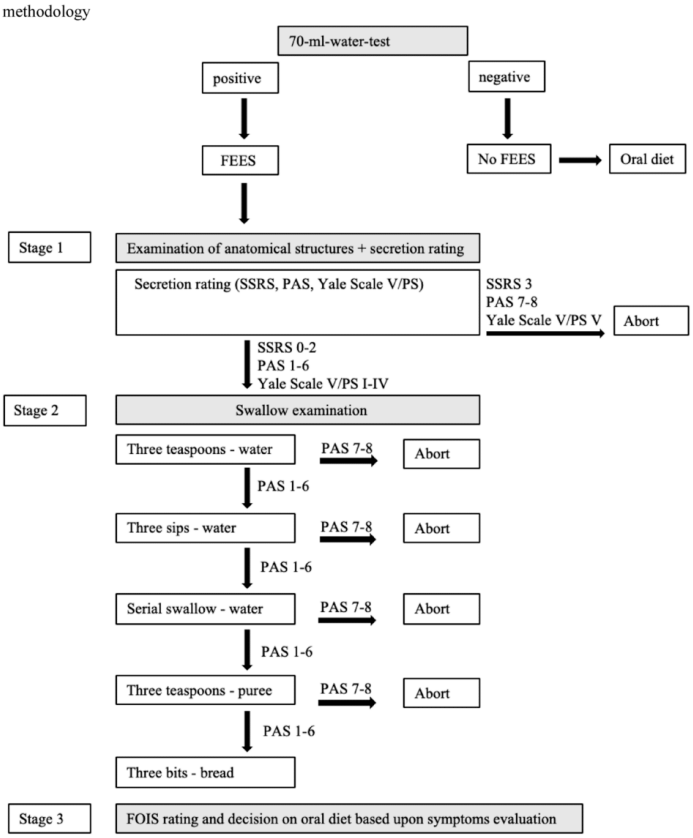

Fig. 3 FEES methodology. *SSRS* secretion severity rating scale, *PAS* penetration- aspiration-scale, *Yale Scale V/PS* yale pharyngeal residue severity rating scale, *V* valleculae, *PS* Piriform sinus)

FOIS-G Pretesting

114 oral diet recommendations built the basis for FOIS-G pretesting by two experienced SLTs with more than 10 years of experience in FEES and dysphagia management. In the first step, a FOIS-G score was assigned to each diet recommendation separate from each other. In a subsequent joint discussion, the assigned scores were discussed by the two SLTs and in cases of deviations a mutual compromise was determined. The agreement between the two SLTs served as the gold standard for validity analysis as well as for the following ratings by six experienced SLTs.

FOIS-G Rating

Six dysphagia experienced SLTs with German as their native language working at various hospital sites in Germany and Austria were recruited for the rating of FOIS-G. Their working experience ranged from 2–19 years (mean 10.5 years). The sole training in the usage of the FOIS-G was the presentation of the scale a week before the actual rating took place. The raters had one week to ask questions and discuss the usage of the scale with the author of this article. All SLTs were blinded about each other's ratings and the pretesting of FOIS-G. For the rating of FOIS-G, the participating SLTs were asked to assign a FOIS-G score to the 114 oral recommendations. A total of 100% of SLTs has conducted the rating. The evaluation of six paired raters were all blinded to each other.

Statistical Analysis

Statistical analyses were performed using SPSS 25.0 statistical software (IBM, SPSS, Inc., Chicago, IL, USA). The calculation of Fleiss kappa and linear weighted Cohen's kappa were carried out with Real Statistics

Resource Pack (www.real-statistics.com), a free add-in software for Microsoft Excel.

Inter-Rater Reliability

Due to the possible agreement between the two SLT's by chance, we used Cohen's kappa statistic, especially a linear weighted Cohen's kappa to attribute more weight on higher disagreements [40]. To calculate inter-rater reliability between paired raters we used percentage agreement and Spearman rank correlation. In addition, to determine the overall agreement between all six raters by subtracting out agreement due to chance, we used Fleiss kappa [40].

Criterion Validity

To evaluate criterion validity, the association between the FOIS-G ratings and BI and MRS was calculated with Spearman rank correlation. Furthermore, we dichotomized the data from BI and MRS with established criteria (MRS score ≤ 3, BI ≤ 75) and used χ^2 and Cramer's V statistic for comparisons. In the original work, the dichotomization was set at ≤ 3 for MRS and ≤ 15 for MBI for moderate disability. For our validation, we set the dichotomization of BI at ≤ 75, which usually represents moderate disability according to Geert et al. 1999 [33]. We expected significant positive correlation of the FOIS with BI and a significant negative correlation of the FOIS with MRS both for dichotomized and non-dichotomized data.

Cross-Validation

Cross-validation between FOIS-G ratings and PAS as well as between FOIS-G ratings and the 70 ml-water-test was calculated with Spearman rank correlation. We expected significant negative correlation of the FOIS-G with the PAS score and a significant positive correlation of the FOIS-G with the 70 ml-water-test.

Results

Inter-Rater Reliability

The agreement of the two SLT's during the pretesting, which was calculated with linear weighted Cohen's kappa, was high ($\kappa = 0.96$, s.e. 0.02). Percentage agreement between all paired raters ranged from 81 to 94%. Spearman rank correlation of all paired raters ranged from 0.96 to 0.99. The overall agreement between all six raters by using Fleiss kappa was high ($\kappa = 0.83$, s.e. 0.01) (Tables 3 and 4).

Criterion Validity

Spearman rank correlation reveals that all stroke measures (MRS, BI) were significantly correlated with FOIS-G (pretesting) score and FOIS-G (average six raters) score on pre-admission, admission to stroke unit, and discharge from stroke unit (Tables 3 and 4).

χ^2 calculation of dichotomized data shows significant associations between FOIS-G (pretesting) score and MRS

Table 2 Clinical and demographic features of 93 stroke patients

Demographic and clinical features	$n = 93$	FOIS ratings after initial FEES						
		1	2	3	4	5	6	7
		22	3	8	1	27	9	23
Mean age ± SD (years)	77.85 ± 10.55	80.18 ± 7.16	82.33 ± 13.70	80.62 ± 8.93	83 ± 0	81.30 ± 7.42	73.11 ± 15.23	71.65 ± 10.96
Sex (%)								
Male	64.52	77	33	50	100	59	67	65
Female	35.48	23	67	50	0	41	33	36
Pathology								
Cerebral infarction	76	20	3	6	0	22	7	18
Cerebral hemorrhage	13	2	0	0	1	5	2	3
Transient ischemic attack	4	0	0	2	0	0	0	2
Mean BI score	32.47	21.81	8.33	12.5	10	32.22	51.11	46.74
Mean MRS score	3.49	3.95	4.33	4.25	5	3.37	3.22	2.87
Mean PAS score	3.85	6.81	2	5	2	4.37	1.78	1.13

BI Barthel Index, *MRS* Modified Ranking Scale, *PAS* Penetration-Aspiration-Scale

Table 3 χ^2, Cramer's V, and Spearman's rho Correlation between the FOIS-G (pretesting) and the BI and MRS scores taken at pre-admission and admission to stroke unit and discharge from stroke unit

Test	χ^2	p	Cramer's V correlation	Spearman rho	p
Pre-admission					
MRS	8.887	0.180	0.309	− 0.329	0.001
Admission					
BI	4.376	0.626	0.217	0.301	0.003
MRS	12.213	0.057	0.362	− 0.366	< 0.001
Discharge					
BI	11.803	0.067	0.356	0.520	< 0.001
MRS	18.563	0.005	0.447	− 0.474	< 0.001

Table 4 χ^2, Cramer's V and Spearman`s rho correlation between the FOIS-G (average six raters) and the BI and MRS Scores taken at pre-admission and admission to stroke unit and discharge from stroke unit

Test	χ^2	p	Cramer's V correlation	Spearman rho	p
Pre-admission					
MRS	17.165	0.579	0.430	− 0.344	0.001
Admission					
BI	15.944	0.661	0.414	0.307	0.003
MRS	23.843	0.202	0.506	− 0.389	< 0.001
Discharge					
BI	21.072	0.333	0.476	0.523	< 0.001
MRS	30.992	0.040	0.577	− 0.497	< 0.001

at discharge from stroke unit ($\chi^2 = 18.563$, $p = 0.005$). FOIS-G (average six raters) score was significantly associated with MRS at discharge from stroke unit ($\chi^2 = 30.992$, $p = 0.040$). With dichotomized data, no association was found between FOIS-G scores and BI (Tables 3 and 4).

Cross-Validation

Spearman rank correlations reveals that the PAS score is significantly correlated with FOIS-G (pretesting) score ($r_s = -0.758$, $p < 0.001$) and FOIS-G (average six raters) score ($r_s = -0.757$, $p < 0.001$).

The 70 ml-water-test could not be performed on all patients due to various post-stroke symptoms such as impaired vigilance, aphasia or speech apraxia. Therefore, we calculated the Spearman rank correlation between FOIS-G scores and 70 ml-water-test scores with a sub sample size of 76 subjects. We found significant correlations between 70 ml-water-test scores and FOIS-G (pretesting) score ($r_s = 0.542$, $p < 0.001$) and FOIS-G (average six raters) score ($r_s = 0.534$, $p < 0.001$).

Discussion

The FOIS has been the most commonly used scale for the rating of the range of oral intake by patients suffering dysphagia and is used both in clinical and in research settings.

This retrospective study aimed at cross-cultural adaptation of the FOIS scale into German (FOIS-G). We conducted the translation by implementing the team approach within the TRAPD-methodology without the interim step of back translation. Even though usually implemented and recommended in cross-cultural adaptation processes of surveys, questionnaires, and self-reported outcome measures [42, 43], the method of back translation, first, does not possess a profound science-based background and, second, does not always ensure an improved quality of the final version [31, 32].

The validation process was based on the study design of the original scale. Not all items used in the original work were included in the validation of the German version due to cross-cultural differences in the implementation of stroke treatment guidelines. The inter-rater reliability was high for both the pretesting by two experienced SLTs and for the rating by six experienced SLTs and presented a significant correlation between all included stroke measures and the FOIS-G. The minimal discrepancy between the two SLTs in pretesting (in only three cases) was due to insecurities concerning the definition of oral intake of patients with regular oral intake parallel to intravenous nutrition. After consultation with the original FOIS author, it was determined that intravenous nutrition is equal to tube-dependent intake.

As expected, we found significant statistical correlations between the FOIS-G and all outcome measures: the MRS, the BI, the PAS score and with the 70 ml-water-test. These results are very similar to the original work despite not fully equal study designs. The FOIS-G inter-rater reliability with $K = 0.96$ between two raters for pretesting as well as the percentage agreement for all six paired raters are high with 81% to 94% for FOIS-G vs. 85% to 95% for the original FOIS. Spearman rank correlation between all raters in FOIS-G is $r_s = 0.96$ to $r_s = 0.99$ (original FOIS $r_s = 0.98$ to $r_s = 0.99$). Overall agreement between all six paired raters for FOIS-G is summed up to $K = 0.83$ (original FOIS $K = 0.86$ to $K = 0.91$).

As for criterion validity without dichotomization the stroke measures (MRS, BI, and 70 ml-water-test) correlated significantly with FOIS-G both in pretesting as well as in the evaluation by six paired raters on pre-admission, at admission and at discharge from stroke unit. As in the original work our dichotomized data for MRS BI and 70 ml-water-test show a significant association between FOIS-G and MRS at discharge both for pretesting as well

as for six average raters. Equivalent to the results of cross-validation of the original FOIS and VFSS a significant correlation between FOIS-G and the PAS score in FEES was found both in pretesting with two raters as well as in rating by all six paired raters. The 70.-ml-water-test in a subsample of 76 subjects shows a significant correlation with FOIS-G.

Even though the cross-cultural adaptation of the Chinese and the Italian version of the FOIS have been conducted in different study settings very strong similarities are found with those results as well: The inter-rater reliability for both Chinese and Italian version are strong (Italian FOIS ICC = 0.99; Chinese FOIS $K = 0.881$, Spearman rank correlation $r_s = 0.972$; Chinese water swallow test $K = 0.844$, Spearman rank correlation $r_s = 0.965$). The Italian FOIS version did not conduct the calculation of criterion validity nor of cross-validation. However, the Chinese results are very similar to the original FOIS and the FOIS-G with a strong correlation found between FOIS and the water swallow test. Furthermore, NIHSS and MBI are also significantly associated with the Chinese FOIS. Cross-validation shows a high association with the Chinese FOIS and the presence of dysphagia and aspiration in VFSS.

Despite the different approaches of the cross-cultural adaptions of the original FOIS scale it is recognizable that all three validated translations show a high inter-rater reliability and, except the Italian version, very strong correlations for criterion validity and cross-validation.

The different study designs are due to the fact that there is no uniform approach to dysphagia management worldwide. In Germany and Italy, the water swallow test is carried out by SLTs, in China by nurses. In addition, there is still no worldwide consensus on which clinical swallow test whether FEES or VFSS should be used as gold standard of instrumental dysphagia diagnostic tool. As consequence, FEES and VFSS are not uniformly used in the same quantity and quality as the gold standard for instrumental dysphagia diagnostics.

The similarity in inter-rater reliability between all three translated versions of the original FOIS is due to the good consensual and criterion validity of the original FOIS scale. As for the cross-validation the fact that the results for both VFSS and FEES are similar in all three translated versions shows that both instrumental tools as well as the FOIS mirror a high validity.

In Germany FEES has become the gold standard of instrumental dysphagia diagnostics being used in more than 70% stroke units [12], whereas VFSS is found only in a few facilities across the country. Validating FOIS-G for FEES adds both to the value of the FOIS and to the FEES examination in research and clinical settings. The results of this study consolidate FEES as an important diagnostic tool in the acute stroke setting as well as in the acute stroke dysphagia management and show the relevance of the implementation of FOIS in everyday clinical practice.

Study Limitations

We did not conduct the inter-rater reliability of FOIS-G for the 70 ml-water-test since at that point of time the FOIS was recorded only for FEES data. This clearly is a limitation to this study as well as the retrospective design of the study. The retrospective characteristic of the study may have caused the negative correlation between the FOIS-G and the BI in dichotomized data contrary to the original work where all stroke measures show a strong association with FOIS in criterion validity for dichotomized data. It is presumable that raising the BI score in a prospective study design prior to the FEES may have resulted in positive results for the correlation between FOIS-G and BI.

With the increasing globalization of the evidence-based medicine, we see, in an ideal case, a uniform description of the results concerning both the transnational clinical patient care and research. Scores are an opportunity to enable and establish international comparability. However, in this context, a thorough validation of each test in the language of each country is an obligatory/irrefutable condition. In the case of the FOIS, the German version, at hand, is only the third translation (besides the Italian and Chinese version [44, 45]) from the 2005 original scale published in English. This circumstance emphasizes, on the one hand, the need for additional, comprehensive translations and validations and, on the other hand, the simultaneous development of new scales in a variety of languages with nominal time, economic and personal associated investments. Along these lines, we support future structural efforts towards a change in paradigm by means of international cooperation in the development of new dysphagia scores and/or the modification of already existent scales.

The design and results of the present study as well as the comparison with existing adaptations show the necessity of a worldwide uniform approach in the design of dysphagia management. The use of validated scales in several languages is an important step in this direction.

Conclusion

FOIS-G was translated according to international translation guidelines and validated by experienced SLTs with German as their native language. It is a valid instrument for the evaluation of functional oral intake of liquids and food by dysphagia patients and can be easily implemented both in clinical and research settings.

Acknowledgements Open Access funding provided by Projekt DEAL.

Funding For this work no financial support or grants have been received.

Compliance with Ethical Standards

Conflict of interest The authors declared that they have no conflict of interest.

Ethical Approval This study was approved by the ethics committee of the University of Giessen (Az. 208/16).

References

1. Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke: incidence, diagnosis, and pulmonary complications. Stroke. 2005;36(1):2756–63. https://doi.org/10.1161/01.STR.0000190056.76543.eb.
2. Altman KW, Yu GP, Schaefer SD. Consequence of dysphagia in the hospitalized patient: impact on prognosis and hospital resources. Arch Otolaryngol Head Neck Surg. 2010;136(8):784–9. https://doi.org/10.1001/archoto.2010.129.
3. Wilson RD. Mortality and cost of pneumonia after stroke for different risk groups. J Stroke Cerebrovasc Dis. 2012;21(1):61–7. https://doi.org/10.1016/j.jstrokecerebrovasdis.2010.05.002.
4. Miller N, Patterson J. Dysphagia: implications for older people. Rev Clin Gerontol. 2014;24:41–57. https://doi.org/10.1017/S0959259813000021X.
5. Barer DH. The natural history and functional consequences of dysphagia after hemispheric stroke. J Neurol Neurosurg Psychiatry. 1989;52:236–41. https://doi.org/10.1136/jnnp.52.2.236.
6. Gottlieb D, Kipnis M, Sister E, Vardi Y, Brill S. Validation of the 50 ml drinking test for evaluation of post-stroke dysphagia. Disabil Rehabil. 1996;18(10):529–32. https://doi.org/10.3109/09638289609166040.
7. Meng NH, Wang TG, Lien IN. Dysphagia in patients with brainstem stroke: incidence and outcome. Am J Phys Med Rehabil. 2000;79(2):170–5. https://doi.org/10.1097/00002060-200003000-00010.
8. Hamdy S. Role of neurostimulation and neuroplasticity in the rehabilitation of dysphagia after stroke. Perspect Swallowing Swallowing Disord. 2010;19(1):3–9. https://doi.org/10.1044/sasd19.1.3.
9. Smithard DG, O'Neill PA, Parks C, Morris J. Complications and outcome following acute stroke: does dysphagia matter? Stroke. 1996;27:1200–4. https://doi.org/10.1161/01.STR.27.7.1200.
10. Mann G, Hankey GJ, Cameron D. Swallowing Function After Stroke: prognosis and prognostic factors at 6 months. Stroke. 1999;30(4):744–8. https://doi.org/10.1161/01.str.30.4.744.
11. Martino R, Pron G, Diamant NE. Screening for oropharyngeal dysphagie in stroke: insufficient evidence for guidelines. Dysphagia. 2000;15(1):19–30. https://doi.org/10.1007/s004559910006.
12. Flader CM, Rosendahl C, Günther T. Leitlinie Konforme Dysphagiediagnostik. Eine repräsentative Befragung von Logopäden an zertifizierten Stroke-Units. Nervenarzt. 2017;88:1168–76.
13. Rosenbek JC, Robbins JA, Roecker EB, Coyle JL, Wood JL. A penetration-aspiration scale. Dysphagia. 1996;11(2):93–8. https://doi.org/10.1007/BF00417897.
14. Murray J, Langmore SE, Ginsberg S, Dostie A. The significance of accumulated oropharyngeal secretions and swallowing frequency in predicting aspiration. Dysphagia. 1996;11(2):99–103. https://doi.org/10.1161/STROKEAHA.113.003048.
15. Crary MA, Mann GD, Groher ME. Initial psychometric assessment of a functional oral intake scale for dysphagia in stroke patients. Arch Phys Med Rehabil. 2005;86(8):1516–20. https://doi.org/10.1016/j.apmr.2004.11.049.
16. Heijnen BJ, Speyer R, Baijens LW, Bogaardt HC. Neuromuscular electrical stimulation versus traditional therapy in patients with Parkinson's disease and oropharyngeal dysphagia: effects on quality of life. Dysphagia. 2005;27(3):336–45. https://doi.org/10.1007/s00455-011-9371-z.
17. Athukorala RP, Jones RD, Sella O, Huckabee ML. Skill training for swallowing rehabilitation in patients with Parkinson's disease. Arch Phys Med Rehabil. 2014;95(7):1374–82. https://doi.org/10.1016/j.apmr.2014.03.001.
18. Wada A, Kawakami M, Liu M, Otaka E, Nishimura A, Liu F, Otsuka T. Development of a new scale for dysphagia in patients with progressive neuromuscular diseases: the Neuromuscular Disease Swallowing Status Scale (NdSSS). J Neurol. 2015;262(10):2225–31. https://doi.org/10.1007/s00415-015-7836-y.
19. Christiaanse ME, Mabe B, Russell G, Simeone TL, Fortunato J, Rubin B. Neuromuscular electrical stimulation is no more effective than usual care for the treatment of primary dysphagia in children. Pediatr Pulmonol. 2011;46(6):559–65. https://doi.org/10.1002/ppul.21400.
20. Bhatt AD, Goodwin N, Cash E, Bhatt G, Silverman CL, Spanos WJ, Jm B, Potts K, Redman R, Allison WA, Dunlap NE. Impact of transcutaneous neuromuscular electrical stimulation on dysphagia in patients with head and neck cancer treated with definitive chemoradiation. Head Neck. 2015;37(7):1051–6. https://doi.org/10.1002/hed.23708.
21. Heijnen BJ, Speyer R, Baijens LW, Bogaardt HC. Neuromuscular electrical stimulation versus traditional therapy in patients with Parkinson's disease and oropharyngeal dysphagia: effects on quality of life. Dysphagia. 2012;27(3):336–45. https://doi.org/10.1007/s00455-011-9371-z.
22. McMicken BL, Muzzy CL, Calahan S. Retrospective ratings of 100 first time-documented stroke patients on the Functional Oral Intake Scale. Disabil Rehabil. 2010;32(14):1165–72. https://doi.org/10.3109/09638280903437238.
23. Salassa JR. A functional outcome swallowing scale for staging oropharyngeal dysphagia. Dig Dis. 1999;17(4):230–4. https://doi.org/10.1159/000016941.
24. Kunieda K, Ohno T, Fujishima I, Hojo K, Morita T. Reliability and validity of a tool to measure the severity of dysphagia: the Food Intake Level Scale. J Pain Symptom Manag. 2013;46(2):201–6. https://doi.org/10.1016/j.jpainsymman.2012.07.020.
25. O'Neil KH, Purdy M, Falk J, Gallo L. The dysphagia outcome and severity scale. Dysphagia. 1999;14:139–45. https://doi.org/10.1007/PL00009595.

26. Smithard DG. Dysphagia management and stroke units. Curr Phys Med Rehabil Rep. 2016;4(4):287–94. https://doi.org/10.1007/s40141-016-0137-2.
27. McGinnis CM, Homan K, Solomon M, Taylor J, Staebell K, Erger D, Raut N. Dysphagia: interprofessional management, impact, and patient-centered care. Nutr Clin Pract. 2019;34(1):80–95. https://doi.org/10.1002/ncp.10239.
28. Survey Research Center. Guidelines for best practice in cross-cultural surveys. Ann Arbor, MI: Survey Research Center, Institute for Social Research, University of Michigan. (2016) https://www.ccsg.isr.umich.edu/. Accessed 04 March 2019
29. European Social Survey. ESS Round 9 Translation Guidelines. London: ESS ERIC Headquarters (2018). https://www.europeansocialsurvey.org/docs/round9/methods/ESS9_translation_guidelines.pdf. Accessed 04 March 2019
30. Behr D. Assessing the use of back translation: the shortcomings of back translation as a quality testing method. Int J Soc Res Methodol. 2017;20(6):573–84. https://doi.org/10.1080/13645579.2016.1252188.
31. Behr, D. Translationswissenschaft und international vergleichende Umfrageforschung: Qualitätssicherung bei Fragebogenübersetzungen als Gegenstand einer Prozessanalyse. GESIS - Leibniz-Institut für Sozialwissenschaften (2019). https://nbn-resolving.org/urn:nbn:de:0168-ssoar-261259. Accessed 04 March 2019
32. Guillemin F, Bombardier C, Beaton D. Cross-cultural adaptation of health-related quality of life measures: literature review and proposed guidelines. J Clin Epidemiol. 1993;46(12):1417–32. https://doi.org/10.1016/0895-4356(93)90142-N.
33. Acquadro C, Jambon B, Ellis D, Marquis P. Language and translation issues. In: Spilker B, editor. Quality of life and pharmacoeconomics in clinical trial. 2nd ed. Philadelphia: Lippincott-Raven; 1996. p. 575–585.
34. McKay RB, Breslow MJ, Sangster RJ, Gabbard SM, Reynolds RW, Nakamoto JM, et al. Translating survey questionnaires: lessons learned. New Dir Eval. 1996;70:93–105. https://doi.org/10.1002/ev.1037.
35. Mann G. MASA, the mann assessment of swallowing ability. New York: Cengage learning; 2002.
36. Antonios N, Carnaby-Mann G, Crary M, Miller L, Hubbard H, Hood K, Sambandam R, Xavier A, Silliman S. Analysis of a physician tool for evaluating dysphagia on an inpatient stroke unit: the modified Mann Assessment of Swallowing Ability. J Stroke Cerebrovasc Dis. 2010;19(1):49–57. https://doi.org/10.1016/j.jstrokecerebrovasdis.2009.03.007.
37. Ohira M, Ishida R, Maki Y, Ohkubo M, Sugiyama T, Sakayori T, Sato T. Evaluation of a dysphagia screening system based on the Mann Assessment of Swallowing Ability for use in dependent older adults. Geriatr Gerontol Int. 2017;17(4):561–7. https://doi.org/10.1111/ggi.12755.
38. Daniels SK, McAdam CP, Brailey K, Foundas AL. Clinical assessment of swallowing and prediction of dysphagia severity. Am J Speech Lang Pathol. 1997;6:17–24. https://doi.org/10.1044/1058-0360.0604.17.
39. Neubauer PD, Rademaker AW, Leder SB. The yale pharyngeal residue severity rating scale: an anatomically defined and image-based tool. Dysphagia. 2015;30(5):521–218. https://doi.org/10.1007/s00455-015-9631-4.
40. Fleiss JL. Statistical methods for rates and proportions. New York: Wiley; 2003.
41. Geert S, Steen C, De Keyser J. Use of the barthel index and modified rankin scale in acute stroke trials. Stroke. 1999;30:1538–41. https://doi.org/10.1161/01.STR.30.8.1538.
42. Schmitt M, Eid M. Guidelines for the translation of foreign-language measurement instruments. Diagnostica. 2007;53:1–2. https://doi.org/10.1026/0012-1924.53.1.1.
43. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. Spine. 2000;25(24):3186–91. https://doi.org/10.1097/00007632-200012150-00014.
44. Zhou H, Zhu Y, Zhang X. Validation of the Chinese Version of the Functional Oral Intake Scale (FOIS) Score in the Assessment of Acute Stroke Patients with Dysphagia. Stud Health Technol Inform. 2017;245:1195–9. https://doi.org/10.3233/978-1-61499-830-3-1195.
45. Battel I, Calvo I, Walshe M. Cross-cultural validation of the italian version of the functional oral intake scale. Folia Phoniatr Logop. 2018;70(3–4):117–23. https://doi.org/10.1159/000490792.

Samra Hamzic MA

Tobias Braun MD

Martin Juenemann MD

Marius Butz Dipl. Psych.

Robert Voswinckel MD

Michael Belly MD

Oliver Vogelbusch MD

Hasan Khilan Cand. Med.

Manfred Kaps MD

Tibo Gerriets MD

5.5. Adjustment of oral diet based on flexible endoscopic evaluation of Swallowing (FEES) in acute stroke patients: a cross-sectional hospital-based registry study[211]

Tobias Braun*, Martin Jünemann*, Maxime Viard, Marco Meyer, Iris Reuter, Mario Prosiegel, Manfred Kaps, Christian Tanislav

*contributed equally

Die Diagnose einer Dysphagie ist in der Akutphase von hoher Bedeutung für Schlaganfallpatienten, da diese Diagnose mit einer erhöhten Mortalität und Morbidität assoziiert ist. Da in der Kohorte der vorherigen Studie ein hoher Anteil an Schlaganfallpatienten enthalten war, führten wir eine entsprechende Subgruppenanalyse durch, um die Auswirkungen der Dysphagie und der Entscheidungen nach FEES-Untersuchung weiter zu untersuchen.

Patienten aus dem Register mit Schlaganfall, die eine FEES-Untersuchung erhalten hatten, wurden in die Subgruppenanalyse eingeschlossen. Es erfolgte eine Analyse bezüglich epidemiologischer Basisparameter, Ausmaß der Behinderung, Diagnose einer Pneumonie, Dauer des stationären Aufenthalts, Notwendigkeit der Intensivbehandlung und maschineller Beatmung.

Insgesamt wurden 152 Patienten in die Subgruppenanalyse eingeschlossen. Das mediane Alter lag bei 73 Jahren, 94 Patienten waren männlich. 125 Patienten (82,2%) hatten einen ischämischen Schlaganfall und 27 (17,8%) eine intrazerebrale Blutung erlitten. Eine oropharyngeale Dysphagie wurde bei 72,4% mittels FEES festgestellt. Patienten mit einer Dysphagie hatten eine höhere Schlaganfallschwere bei Aufnahme (medianer NIHSS-Score 11 [IQR 6-17] vs. 7 [IQR 4-12], p=0,013; medianer mRS-Score 5 [IQR 4-5] vs. 4 [IQR 3-5], p=0,012). Auch die Mortalität während des stationären Aufenthalts bei Patienten mit Dysphagie war höher im Vergleich zu Patienten ohne Dysphagie (7,2% vs. 0%; p=0,107). Die FEES-Untersuchung zeigte, dass lediglich 30,9% der Patienten eine Ernährungsweise hatten, die ihren Fähigkeiten entsprach. Bei den übrigen Patienten wurde die Ernährungsart angepasst. Eine Anpassung der Ernährungsart war assoziiert mit einem besseren klinischen Outcome bei Entlassung (mRS 4,5 [IQR 3-5] vs. 4 IQR [3-4]; p=0,006), einer verminderten Notwendigkeit für eine maschinelle Beatmung länger als 24 Stunden (31,9 vs. 15,2%; p=0,028), einer kürzeren Dauer des Krankenhausaufenthalts (22 Tage [IQR 13-30] vs. 16 Tage [11-25]; p=0,044), einer geringeren Pneumonierate (57,4% vs. 33,3%; p=0,007) sowie einer geringeren Mortalität (12,8% vs 1,9%; p=0,011).

Da die klinische Untersuchung eine stille Aspiration nicht aufdecken kann, scheint die FEES beim Schlaganfall besser geeignet, um Risikopatienten zu identifizieren und entsprechende Maßnahmen zu ergreifen. Dies könnte zu einem besseren funktionellen Outcome, zu geringeren Pneumonieraten und einer geringeren Mortalität beitragen. Unsere Ergebnisse legen auch nahe, dass selbst in einer spezialisierten Schlaganfallabteilung das Bewusstsein für Schluckstörungen nicht ausreichend ist. Die niederschwellige Durchführung einer FEES-Untersuchung in der akuten Phase eines Schlaganfalls kann durch Anpassung der Ernährung an die Fähigkeiten des Patienten potenziell dazu beitragen, schwere Komplikationen zu vermeiden.

Braun et al. BMC Neurology (2019) 19:282
https://doi.org/10.1186/s12883-019-1499-8

BMC Neurology

RESEARCH ARTICLE Open Access

Adjustment of oral diet based on flexible endoscopic evaluation of swallowing (FEES) in acute stroke patients: a cross-sectional hospital-based registry study

Tobias Braun[1,2*†], Martin Juenemann[1†], Maxime Viard[1], Marco Meyer[1,3], Iris Reuter[1], Mario Prosiegel[4], Manfred Kaps[1] and Christian Tanislav[1,3]

Abstract

Background: Diagnosing dysphagia in acute stroke patients is crucial, as this comorbidity determines morbidity and mortality; we therefore investigated the impact of flexible nasolaryngeal endoscopy (FEES) in acute stroke patients.

Methods: The FEES investigation as performed in acute stroke patients treated at a large university hospital, allocated as a standard procedure for all patients suspected of dysphagia. We correlated our findings with baseline data, disability status, pneumonia, duration of hospitalisation, necessity for mechanical ventilation and treatment on the intensive care unit. The study was designed as a cross-sectional hospital-based registry.

Results: We investigated 152 patients. The median age was 73; 94 were male. Ischemic stroke was diagnosed in 125 patients (82.2%); 27 (17.8%) suffered intracerebral haemorrhage.
Oropharyngeal dysphagia was diagnosed in 72.4% of the patients, and was associated with higher stroke severity on admission (median NIHSS 11 [IQR 6–17] vs. 7 [4–12], $p = .013$; median mRS 5 [IQR 4–5] vs. 4 [IQR 3–5], $p = .012$). Short-term mortality was higher among patients diagnosed with dysphagia (7.2% vs. 0%, $p = .107$). FEES examinations revealed that only 30.9% of the patients had an oral diet appropriate for their swallowing abilities. A change of oral diet was associated with a better outcome at discharge (mRS; $p = .006$), less need of mechanical ventilation ($p = .028$), shorter period of hospitalisation ($p = .044$), and lower rates of pneumonia ($p = .007$) and mortality ($p = .011$).

Conclusion: Due to the inability of clinical assessments to detect silent aspiration, FEES might be better suited to identify stroke patients at risk and may contribute to a better functional outcome and lower rates of pneumonia and mortality. Our findings also point to a low awareness of dysphagia, even in a specialised stroke centre.
FEES in acute stroke patients helps to adjust the oral diet for the vast majority of stroke patients (69.1%) based on their swallowing abilities, potentially avoiding severe complications.

* Correspondence: tobias.braun@neuro.med.uni-giessen.de
†Tobias Braun and Martin Juenemann contributed equally to this work.
[1]Department of Neurology, University Hospital Giessen and Marburg, Klinikstrasse 33, 35392 Giessen, Germany
[2]Department of Neurology, Justus Liebig University, Klinikstrasse 33, 35392 Giessen, Germany
Full list of author information is available at the end of the article

Background

Dysphagia occurs in the course of many neurological diseases and frequently determines the outcome [1] with stroke being the most common cause. Up to 80% of stroke patients suffer from dysphagia, depending on the choice of diagnostics tests used (screening tests, comprehensive swallowing assessment by SLT and instrumented methods, such as VFS or FEES) [2]. Pneumonia due to dysphagia is the leading cause of death in stroke patients [3]. The risk for pneumonia increases up to 11.5-fold in stroke patients, if penetration or aspiration of secretions, food or fluids is present [2]. Hyperthermia, that can be caused by the pneumonia-associated fever, is known to be associated with a worse functional outcome in stroke [4]. Another known factor associated with a worse outcome in stroke patients is new or pre-existing malnutrition, which can also be caused by dysphagia [5]. Moreover, dysphagia is an independent predictor of disability and poor outcome, increased mortality, morbidity and markedly reduced quality of life in stroke [8–11]. Dysphagia not only leads to further complications in stroke patients, but its resultant long-term healthcare costs underline its socioeconomic relevance [6, 7].

Thus, one of the positive effects of stroke unit therapy seems to be an early diagnosis of dysphagia and the improvement of the swallowing function, thereby preventing pneumonia, malnutrition and dehydration.

Diagnostic tools for dysphagia include a screening examination, comprehensive swallowing examination (CSE) performed by physicians or speech and language therapists (SLT) as well as instrumented methods, such as videofluoroscopy of swallowing (VFS) or flexible endoscopic evaluation of swallowing (FEES). FEES combines many advantages in the clinical routine, as it is a bedside procedure without radiation exposure; enables the evaluation of saliva handling; it may be performed in uncooperative or unconscious patients; and it can easily be repeated. Moreover, swallowing is assessed by FEES in a more "natural" way than VFS, as the latter requires a contrast media and cued swallowing (to reduce radiation dosage). In this context, FEES might be an important tool for identifying patients at risk and may ultimately help improve functional outcome by adjusting patients' oral diet.

We recently published a paper on the use of FEES and adjusting the oral diet in neurological patients. In this analysis, we were able to demonstrate a lower rate of pneumonia an a lower mortality, when adjusting the oral diet [12]. This is a subgroup analysis for acute stroke patients. As mentioned above, dysphagia is very common in stroke patients, puts those patients at a high risk of complications, increases mortality and leads to a longer length of hospitalisation. Furthermore, outcome parameters, such as the National Institute of Health Stroke Scale (NIHSS) and the modified Rankin-scale (mRS) are routinely assessed in all stroke patients. Therefore, the aim of the current study was to analyse the impact of FEES and adjustment of the oral diet based on those findings in the management of acute stroke patients. The study was designed as a cross-sectional hospital-based registry.

Methods

The study was done in a large German university hospital. As a part of routine care delivery for patients hospitalised for acute stroke, FEES was performed in case of a pathologic bedside screening procedure, performed by nurses or SLTs. In our department, we use the Gugging Swallowing Screen (GUSS) [13]. If the patient passed the GUSS, no FEES was performed and full oral diet was chosen. If the GUSS indicated possible dysphagia, the patient underwent a CSE by an SLT and FEES by a team consisting of a SLT and a neurologist. FEES was also performed if a patient showed signs of pharyngeal dysphagia during hospitalisation (e.g. wet voice, coughing when drinking, etc.) and if a patient developed signs of infection (productive cough, elevated inflammatory markers). The signs of dysphagia were reported by nurses, SLTs or the treating physicians. The patients with signs of dysphagia were discussed among the "dysphagia experts" of our department and indication for FEES was confirmed; oral diet prior to FEES was chosen as instructed by the GUSS or by clinical judgement of the treating physician. For quality control reasons, our findings gathered from examinations were documented systematically. All FEES were performed in a standardised manner by experienced physicians. The screening process is depicted in Fig. 1.

Patients

All stroke patients treated in our department from January 2014 to September 2016 in whom FEES was performed were documented in a standardised manner. Approximately 800 patients per year are discharged from our hospital with the diagnosis of a stroke. Data documented in the database included age, sex, length of stay in hospital, stroke entity (ischemic stroke vs. primary haemorrhage), stroke aetiology (TOAST-criteria in ischemic stroke), NIHSS and mRS on admission and at discharge, localisation of ischemic lesion (left/right hemisphere, bilateral infarctions, brain stem), ischemic vascular territory, presence of risk factors in ischemic stroke

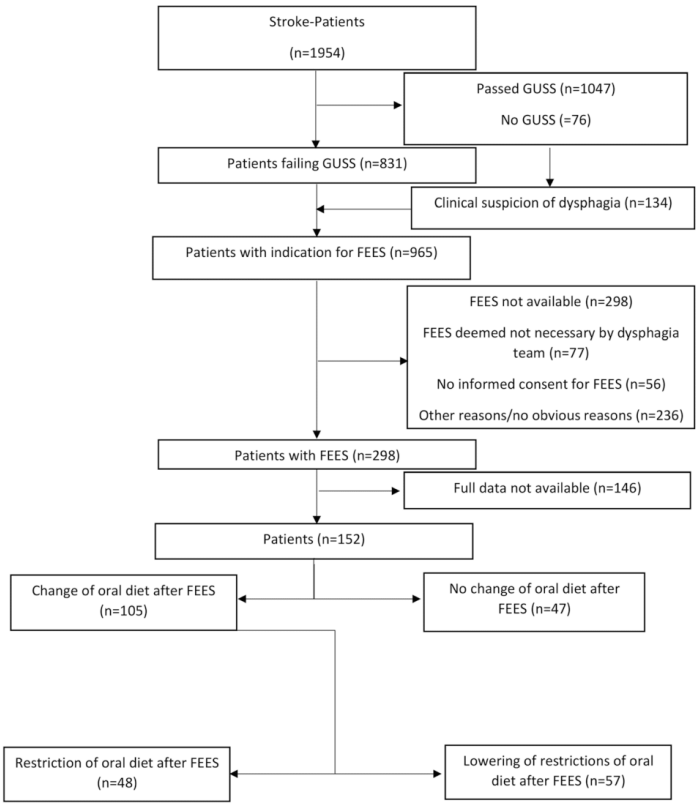

Fig. 1 Screening process and decisions after FEES in patients

(hypertension, atrial fibrillation, diabetes mellitus, hyperlipidaemia, tobacco smoking, cardiovascular disease, previous stroke), occurrence of pneumonia at any given point during hospitalisation (determined by the treating physician due to clinical signs of pneumonia, elevated inflammatory markers in the blood and chest X-ray), treatment on intensive care unit, necessity of intubation and mechanical ventilation lasting longer than 24 h (excluded from this item were patients intubated for surgery, such as decompressive craniotomy in cerebellar infarction or those who were preclinically intubated and promptly extubated), mortality, presence of dysphagia (as determined by the FEDSS, see below) and type of oral intake (before and after FEES; as determined by the FOIS, see below). For acquisition and use of data for scientific analyses, ethical approval was obtained from the local ethical committee (protocol number 208/16). All patients documented in the database were selected for the current analysis.

Fees

FEES is a videoendoscopic nasolaryngeal swallowing study. We performed FEES following a standardised FEES® protocol, according to Langmore [14]: after applying decongestants (Xylometazolin) and local anaesthesia of the nasal duct using 2% Lidocaine-gel, a small endoscope (about 4 mm in diameter) was introduced through the inferior nasal meatus and the nasopharynx in the oropharynx. The swallowing of saliva and different consistencies of food and liquids and penetration, aspiration, localisation and extent of residues, as well as patients' reactions (such as coughing), were visualised and documented. By definition, penetration is entering of any material into the airway above the level of the vocal folds, and aspiration is

entering of any material below the level of the vocal folds [15]. In the first step of the procedure, anatomical changes, handling of saliva and the movement of swallowing-related structures were tested, then pudding-thick consistency (thickened water), normal water and solid food were introduced. For every consistency, we first used a teaspoon, then a table spoon and in case of water, the patient was asked to take a normal swallow from a cup. For better visualisation, the consistencies were dyed blue, using food colour. All consistencies were applied three times. If a consistence appeared unsafe to test, we skipped it; the consistence was rated as unsafe, if it entered the airway to the level of the vocal folds without ejection from the airway or any aspiration (score 5–8 on Rosenbek's Penetration-Aspiration-Scale [16]). In the context of this research manuscript, we defined a "relevant dysphagia" as an oropharyngeal dysphagia with a score of 3–8 on Rosenbek's Penetration-Aspiration-Scale, as this exposes the patient to the risk of pneumonia. Using the findings in FEES, the appropriate oral diet was chosen for the patient. Based on the pathophysiology found in FEES, compensatory and rehabilitative measures to treat dysphagia were carried out as described by Daniels and co-workers [17]. All FEES procedures were performed or supervised by an experienced investigator and lasted about 10 min each.

Outcome measurements

Oral intake and dysphagia severity were measured by use of the functional oral intake scale (FOIS) and the Fiberoptic Endoscopic Dysphagia Severity Score (FEDSS), respectively:

FOIS is a seven-tiered scale ranging from 1 = no oral intake at all (NPO = nil per os) to 7 = full oral intake without restrictions (Table 5) [18]. For easier readability, the data of the functional oral intake scale were categorised in either NPO (FOIS = 1), tube dependency with at least some oral intake (FOIS 2–3), patients without tube dependency with dietary restrictions (single consistency, special preparations or limited specific food) (FOIS =4–6) and oral diet without restriction (FOIS = 7). Restriction of the oral diet was defined as a negative change on the FOIS, whereas lowering of restrictions of the oral diet was defined as a positive change. FOIS was documented prior to and after FEES.

There is no standardised way of defining the overall severity of dysphagia. In our department, we use the FEDSS-scale developed by Dziewas and co-workers [19]. The FEDSS is a six-tiered scale originally designed for use in stroke patients (Table 6). All parameters were recorded in a standardised way.

For evaluating the value of performing FEES in neurological patients, the following parameters were correlated with baseline data and dependent factors:

- Dysphagia as defined by a FEDSS score of ≥2
- The oral intake status as calculated by the FOIS and its overall change and type of change after FEES

Statistical analyses

Absolute and relative frequencies were calculated based on cross-tables. For comparing relative frequencies, we used a two-tailed Fisher's exact test. Continuous variables were analysed by calculating the median value and the interquartile range (IQR; 25% percentile and 75% percentile). Nonparametric non-paired data were analysed using the Mann–Whitney U-test and paired data using the Wilcoxon-test. Binary logistic regression analysis was used to identify factors associated with the item "change in oral diet". All statistical analyses were performed using SPSS, release version 22.0 (SPSS©, Inc., IBM Company, 2015, Chicago-IL).

Results

Patients' characteristics

173 FEES were performed in 152 stroke patients. In 19 (12.5%) patients, the procedure was repeated at least once and their data were only analysed once. In order to prevent data distortion, only the results of the first examination were included in the analysis of patients who received more than one FEES. 94 patients (61.8%) were male and the overall median age was 73 years (IQR 61.25–81 years). 119 patients were older than 60 years (78.3%). 125 patients (82.2%) were diagnosed with ischemic stroke and 27 (17.8%) with primary haemorrhage. 61 patients (48.8%) were treated on the intensive care unit. 62 patients (40.8%, or 26.8% when excluding intensive care patients) were diagnosed with pneumonia and 8 patients (5.3%) died during hospitalisation. Initially, 76 patients (49%) had no oral intake (NPO), 12 patients (7.8%) were tube dependent with at least some oral intake (FOIS 2–3), 45 patients (29.6%) needed no feeding tube but had dietary restrictions intake and 19 patients (12.3%) oral intake without restrictions. Among the patients with NPO or that were tube dependent with some oral intake, 65 patients (42.8%) had a nasogastric feeding tube prior to FEES and 2 patients (1.3%) had a PEG-tube. 31 patients (20.3%) needed intubation with a length of mechanical ventilation of 88 h (IQR 23–479; median 193 h [IQR 67.5–496.5], when excluding ventilation lasting less than 24 h). Of these patients, 11 were intubated preclinically and 13 during the first 6 h in our

hospital. We were unable to reconstruct the reason for intubation in a sufficient number of patients from our data.

Patients' characteristics are presented in Table 1. Patients' characteristics for the subgroup of ischemic stroke patients can be found in the Additional file 1: Table S1.

FEES examination

No side effects occurred, such as laryngospasm, syncope or epistaxis.

The median FEDSS in the entire study population was 4 (IQR 1–6) and the median time from admission to first FEES was 6 days (IQR 3-11 days). FEES identified 110 (72.4%) patients with dysphagia (FEDSS 2–6). A diet modification was indicated in 105 patients (69.1%) with restriction of oral diet in 48 patients (31.6%) and lowering of restrictions in 57 (37.5%). NPO was indicated for the majority of patients (76.6% in this subgroup) without change in oral diet. 8 patients (5.3%) died during hospitalisation; all of them suffered from dysphagia. NIHSS and mRS on admission and at discharge were higher in dysphagic patients than non-dysphagic patients (admission: median NIHSS 11 [IQR 6–17] vs 7 [4–12], $p = .013$; median mRS 5 [IQR 4–5] vs. 4 [IQR 3–5], $p = .012$; discharge: median NIHSS 7 [IQR 4–12] vs 6 [3–11], $p = .05$; median mRS 4 [IQR 3–5] vs. 4 [IQR 2–4], $p = .002$). The outcome at discharge (mRS) in relation to the FEDSS is summarized in Fig. 2. Results are

Table 1 Baseline characteristics in stroke patients with normal swallowing function vs. patients with relevant dysphagia. Statistically significant p-levels are printed in bold

	Total cohort ($n = 152$)	Normal swallowing function ($n = 42$)	Relevant dysphagia ($n = 110$)	*P*
Sex				
Male	94 (61.8%)	25 (59.5%)	69 (62.7%)	0.427
Age median (IQR)	73 (61.25–81)	71 (58.5–80)	74 (63–81)	0.198
Stroke entity				
ischemic stroke	125 (82.2%)	34 (81%)	91 (82.7%)	
primary haemorrhage	27 (17.8%)	8 (19%)	19 (17.3%)	
Stroke severity on admission				
NIHSS on admission; median (IQR)	10 (5–15.5)	7 (4–12)	11 (6–17)	**0.013**
mRS on admission; median (IQR)	4 (3–5)	4 (3–5)	5 (4–5)	**0.012**
Stroke severity at discharge				
NIHSS at discharge; median (IQR)	6 (3–11)	4 (1–9.5)	7 (4–12)	**0.05**
mRS at discharge; median (IQR)	4 (3–5)	4 (2–4)	4 (3–5)	**0.002**
Time from admission to first FEES in days (median, IQR)	6 (3–11)	6 (2–10.25)	6 (3–11)	0.497
Length of stay in hospital in days (median, IQR)	17 (12–27.75)	15.5 (11.75–25.25)	18 (12–29)	0.225
Intensive care unit	61 (48.8%)	14 (33.3%)	47 (42.7%)	0.378
Necessity for intubation & mechanical ventilation lasting longer than 24 h	29 (19.1%)	4 (9.5%)	25 (22.7%)	**0.023**
Pneumonia	62 (40.8%)	16 (38.1%)	46 (41.8%)	0.715
Death	8 (5.3%)	0	8 (7.2%)	0.107
PEG procedure	34 (22.4%)	8 (19%)	26 (23.6%)	0.665
Diet after FEES				
No change in oral diet	47 (30.9%)	7 (16.7%)	40 (36.4%)	**0.019**
Change in oral diet	105 (69.1%)	35 (83.3%)	70 (63.6%)	**0.019**
Restriction	48 (31,6%)	1 (2.4%)	47 (42.7%)	**< 0.001**
Lowering of restrictions	57 (37,5%)	34 (81%)	23 (20.9%)	**< 0.001**

IQR: interquartile range
NIHSS: National institute of Health Stroke Scale
mRS: Modified Rankin-Scale
PEG: percutaneous endoscopic gastrotomy tube

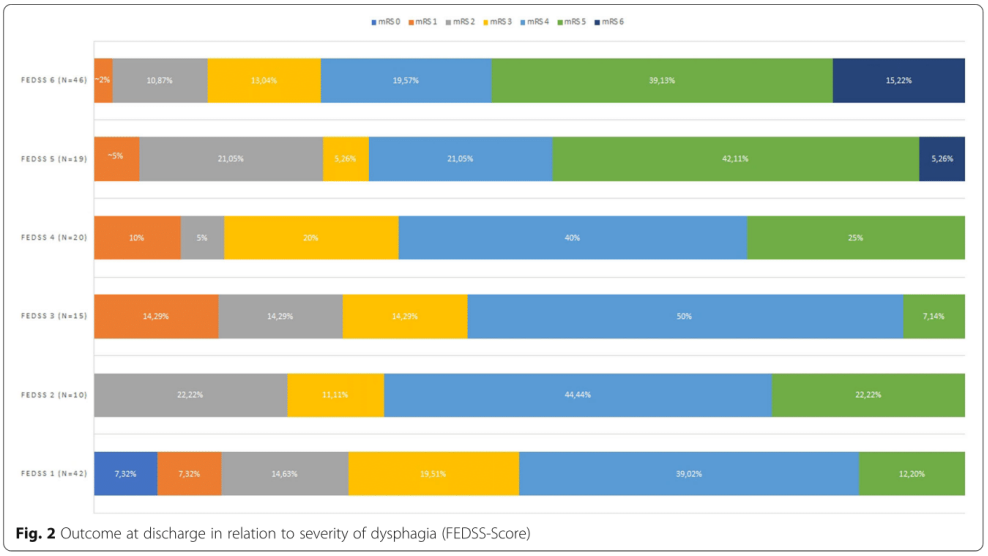

Fig. 2 Outcome at discharge in relation to severity of dysphagia (FEDSS-Score)

summarized in Table 1. The results for the subgroup of ischemic stroke patients can be found in the Additional file 1: Table S1.

Differences in patients with and without change in oral diet

For patients that needed diet changes, the length of stay was shorter (median 16 days [IQR 11–25] vs. 22 days [IQR 13–30], $p = .027$), intubation and mechanical ventilation were less frequently indicated (15.2 vs. 31.9%, $p = .028$) and pneumonia as well as mortality rates were lower (pneumonia; 33.3% vs. 57.4%, $p = .007$; mortality: 1.9% vs 12.8%, $p = .011$). At discharge, mRS was lower in patients with diet changes (median 4 [IQR 3–4] vs. 4.5 [3–5], $p = .006$). A comparison of the intraindividual difference of mRS on admission and at discharge revealed a better functional outcome in patients with a change in oral diet ($p = .001$); we observed no better outcome in patients without a change in oral diet ($p = .583$). Results are summarised in Table 2 and Additional file 1: Table S2 (ischemic stroke patients only).

Binary logistic regression analysis revealed a lower odds-ratio associated with a change of oral diet for pneumonia (Table 3) and intubation (Table 4). The results for the subgroup of ischemic stroke patients can be found in Additional file 1: Table S3 and Table S4.

Discussion

In 72% of our stroke patients, FEES unveiled a relevant dysphagia, leading to an adjustment of oral diet. In those patients, we observed a better functional outcome at discharge and fewer complications, such as the need for mechanical ventilation, a lower mortality rate and a lower rate of pneumonia.

The most alarming result is that only 30.9% of our patients had an appropriate oral diet for their swallowing abilities prior to FEES, meaning more than two thirds of our patients needed adjustment of their oral diet. This demonstrates low awareness of dysphagia and emphasises the need for instrumental diagnostics with a low threshold, proving that extensive clinical expertise avoids significant complications. Screening and CSE are necessary, but unable to detect all kinds of relevant swallowing disturbances, especially silent aspiration. FEES seems to be a more reliable tool than screening and CSE, as it revised the diet strategy suggested by screening and CSE in the majority of patients. Our results underline the necessity of performing FEES at a low threshold in the majority of stroke patients, irrespective of clinical examination and screening tests. This would be in accordance with intentions to change national guidelines as suggested by Lindner-Pfleghar and co-workers [20]. FEES is a safe, fast and reliable tool, as we observed no side effects in about 1730 min of examination. In our cohort, the median time from stroke to FEES was 6 days. As we found no side effects from FEES-examination and the patients' diet was

Table 2 Differences in baseline characteristics between stroke patients with and without change in the oral diet. Statistically significant p-levels are printed in bold

	Total cohort (n = 152)	No change in oral diet (*n* = 47)	Change in oral diet (*n* = 105)	*P*
Sex				
Male	94 (61.8%)	30 (63.8%)	64 (61%)	0.857
Age median (IQR)	73 (61.25–81)	75 (65–79)	72 (61–81.5)	0.657
Stroke entity				
ischemic stroke	125 (82.2%)	38 (80.9%)	87 (82.9%)	
primary haemorrhage	27 (17.8%)	9 (19.1%)	18 (17.1%)	
Stroke severity on admission				
NIHSS on admission; median (IQR)	10 (5–15.5)	11 (5.5–17.5)	9 (5–14)	0.237
mRS on admission; median (IQR)	4 (3–5)	5 (4–5)	4 (3–5)	0.087
Stroke severity at discharge				
NIHSS at discharge; median (IQR)	6 (3–11)	8 (3–13.5)	6 (3–10)	0.172
mRS at discharge; median (IQR)	4 (3–5)	4.5 (3–5)	4 (3–4)	**0.006**
Time from admission to first FEES	6 (3–11)	6 (3–13)	6 (2–10)	0.297
Length of stay in hospital in days (median, IQR)	17 (12–27.75)	22 (13–30)	16 (11–25)	**0.027**
Intensive care unit	61	22 (46.8%)	31 (29.5%)	**0.044**
Necessity for intubation & mechanical ventilation lasting longer than 24 h	31 (20.4%)	15 (31.9%)	16 (15.2%)	**0.028**
Pneumonia	62 (40.8%)	27 (57.4%)	35 (33.3%)	**0.007**
Death	8 (5.3%)	6 (12.8%)	2 (1.9%)	**0.011**
PEG procedure	34 (22.4%)	13 (27.7%)	21 (20%)	0.3

IQR: Interquartile range
NIHSS: National Institute of Health Stroke Scale
mRS: Modified Rankin-Scale
PEG: Percutaneous endoscopic gastrotomy tube

changed in the majority of patients, we recommend using FEES early in the acute phase of stroke unit treatment. Recently, a large FEES-registry study with various neurological diseases was published. The results of this study also confirm the safety of FEES, even when performed by an inexperienced investigator. In this study, the diet was adjusted in approximately about 50% of the patients after the FEES-examination [21].

When adjusting oral diet based on our findings in FEES, we observed in our patients a better outcome, a reduced need of intubation and mechanical ventilation, a lower pneumonia rate, lower mortality and a shorter period of hospitalisation. In a meta-analysis, Steele and co-workers reported a significant reduction in penetration and aspiration when thickening fluids [22]; this might be one factor explaining our results. One other factor contributing to a better outcome might be the increased mobility of patients after removal of a nasogastric feeding tube or an intravenous canulae for parenteral feeding, heightening the effects of physiotherapy. In case of a change in oral diet, the risk of pneumonia or intubation was reduced. Our findings underline the value of FEES in finding a safe oral diet for stroke patients.

Table 3 Binaryl logistic regression analysis for pneumonia. Statistically significant p-levels are printed in bold

	P	Odds-Ratio	95%- Confidence interval
Age above 60	0.367	0.683	0.299–1.563
mRS on admission ≥3	0.897	0.936	0.342–0,256
Change of oral diet	**0.007**	0.362	0.173–0.754
Intubation	0.789	1.125	0.476–2.658
Constant	0.042		

Dysphagic patients had a higher NIHSS and mRS on admission and at discharge. This is in agreement with results by Dziewas and co-workers, who showed that patients with a NIHSS > 3 had signs of penetration and aspiration [19]. Warnecke and co-workers showed that the degree of dysphagia is predictive of functional outcome three months after the initial stroke [9]. Hence, the functional deficit seems to be predictive of dysphagia and vice versa.

Dysphagia was more often diagnosed in right hemispheric ischemia. Teismann and co-workers could

Table 4 Binary logistic regression analysis for intubation

	P	Odds-Ratio	95%- Confidence interval
Age above 60	0.511	0.716	0.264–1.940
mRS on admission ≥3	0.340	2.132	0.450–10.094
Change of oral diet	0.051	0.421	0.176–1.005
Pneumonia	0.808	1.113	0.470–2.633
Constant	< 0.001		

visualize a time-dependent cortical activation of the hemispheres during swallowing using magnetencephalography; during the oral phase of swallowing there was a predominantly left-hemispheric activation, whereas right-sided activation was apparent during the pharyngeal phase of swallowing [23]. As we detected penetration and aspiration (occurring during the pharyngeal phase of swallowing) more often in our patients in right-hemispheric ischemia, our data are in accordance with these findings. Right-sided brain lesions are also associated with neglect and lack of awareness, disposing patients to aspiration [24, 25]. This might be an additional explanation for our findings.

As far as we know, only one study has been published on the effect of FEES about the functional outcome in stroke patients [26]. Bax and co-workers found a reduction in the pneumonia rate and a higher rate of a normal diet at discharge after FEES implementation than before the procedure. The length of stay in their study was longer than in ours, and there were no differences in mortality when FEES was performed routinely. However, this study had potential flaws: (i) the authors compared their patients with a historical control group; (ii) in both groups, the majority of patients were not examined via FEES; and (iii) in terms of functional outcome, the scores of the commonly used NIHSS and mRS at discharge were not reported. Thus, there is no evidence on the impact of FEES on the neurological functional outcome based on these study results.

Our study shows associations between adjusting the diet based on FEES findings and the functional neurological outcome, necessity for intubation and the rate of pneumonia and mortality. Our study design does not allow us to differentiate whether our results regarding better outcomes and fewer complications are based on our intervention (adjustment of oral diet based on FEES-findings) or fewer deficits of the patients. In our opinion, this effect could only be demonstrated by a randomised-controlled trial with patients receiving FEES or no FEES. As we have demonstrated in our cohort that more than two thirds of patients lacked an oral diet, that suited their swallowing abilities. It seems questionable to design a trial that withholds a potentially beneficial diagnostic test to one half of the study population. Potential selection bias of a large number of intensive care patients needs consideration when interpreting our results; as these patients are more severely affected by stroke, this explains the median mRS of 4. Our findings would therefore overestimate the number of neurological patients affected by dysphagia in this context, which might explain the high frequency of pneumonia compared to other studies [27]. Another circumstance to bear in mind when interpreting our results are the effects of rehabilitatory and compensatory strategies that were chosen based on FEES findings. Those strategies might also contribute to fewer complications and better patient outcomes, meaning the change in oral diet might not be the single factor for our results. However, all patients were treated by the same SLTs and when the patient was able to use those techniques, he or she was trained accordingly and instructed to use them. Therefore, the effects of rehabilitatory and compensatory techniques might impact our results. However, these effects should also be present in the group without change in oral diet, as they were also instructed to use these techniques. The long period of 6 days from admission to FEES can be mainly attributed to the large number of intensive care patients, as those patients could only undergo FEES after end of sedation, mechanical ventilation and extubation. These are the study's main limitations; however, the study design represents the clinical routine with a pre-selection of patients by using a screening followed by an instrumented diagnostic. The study's biggest limitation is the lack of a control group, reducing the validity of our results. Because of ethical reasons, we used no control group (without FEES), as - in our opinion - the risk of pneumonia and pneumonia-related death would have been too high. As discussed above, a randomised-controlled trial would be necessary, to clearly demonstrate the effects of FEES.

Conclusions

FEES can better identify acute stroke patients at risk than screening for dysphagia or CSE due to its ability to detect silent aspiration. It is a safe and fast procedure that led to an adjustment of oral diet in roughly two out of three patients, with potential positive consequences for the overall clinical outcome by avoiding pneumonias or mechanical ventilation. Based on our data, and despite the need for large-scaled and randomised-controlled studies, we recommend the use of FEES in stroke patients at a low threshold.

Appendix 1

Table 5 Functional oral intake scale [18]

1	Nothing by mouth (NPO)
2	Tube dependent with minimal attempts of food or liquid
3	Tube dependent with consistent oral intake of food or liquid
4	Total oral diet of a single consistency
5	Total oral diet with multiple consistencies, but requiring special preparation or compensations
6	Total oral diet with multiple consistencies without special preparation, but with specific food limitations
7	Total oral diet with no restrictions

Appendix 2

Table 6 FEDSS-Score [19]

Score		Main findings
6	Handling of secretions/Saliva	Penetration or Aspiration
5	Puree consistency	Penetration/aspiration without or insufficient protective reflex
4		Penetration/aspiration with sufficient protective reflex
4	Liquids	Penetration/aspiration without or insufficient protective reflex
3		Penetration/aspiration with sufficient protective reflex
2	Soft solid food	Penetration/aspiration or massive residues in valleculae or piriforms
1		No penetration/aspiration and no more than mild to moderate residues in valleculae or piriforms

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12883-019-1499-8 .

Additional file 1: Table S1. Differences in baseline characteristics between patients with normal swallowing function versus those with clinically relevant dysphagia in the subgroup of patients with ischemic stroke. **Table S2.** Differences in baseline characteristics between stroke patients with and without change in oral diet in the subgroup of patients with ischemic stroke. **Table S3**. Binaryl logistic regression analysis for pneumonia in ischemic stroke patients. **Table S4**. Binary logistic regression analysis for intubation in ischemic stroke patients.

Abbreviations

BSE: Bedside screening examination; CSE: Comprehensive swallowing examination; CT: Computed tomography; FEDSS: Fiber endoscopic dysphagia severity scale; FEES: Flexible endoscopic evaluation of swallowing; FOIS: Functional oral intake scale; GUSS: Gugging Swallowing Screen; ICU: Intensive care unit; IQR: Interquartile range; MRI: Magnetic resonance imaging; mRS: Modified Rankin-scale; NIHSS: National Institute of Health stroke scale; NPO: Nil per os (no oral intake); PEG: Percutaneous endoscopic gastrostomy tube; SLT: Speech and language therapist; VFS: Videofluoroscopy of swallowing

Acknowledgements

The authors thank Kerstin Ulmrich-Braun for proof-reading the manuscript.

Authors' contributions

TB, MJ, MK and CT: Conceptualisation. TB, MV, MM and IR: FEES examinations. TB, MJ and MP: Analysis of data and statistics. TB, MJ and CT: Preparation of original draft. All authors: Review and editing; ICMJE criteria for authorship read and agree with manuscript results and conclusions.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the article. The data that support the findings of this study are not publically available due to local medical data protection policies.

Ethics approval and consent to participate

For the data acquisition and the use of findings for scientific analyses, an ethical approval was obtained from the local ethical committee (Justus-Liebig University, protocol number 208/16). The ethical committee waived the need for the patients' consent to participate.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Neurology, University Hospital Giessen and Marburg, Klinikstrasse 33, 35392 Giessen, Germany. [2]Department of Neurology, Justus Liebig University, Klinikstrasse 33, 35392 Giessen, Germany. [3]Department of Neurology/Geriatrics, Diakonie Klinikum Jung-Stilling, Wichernstraße 40, 57074 Siegen, Germany. [4]Lecturer at Faculty of Languages and Literatures, Department I, Ludwig-Maximilians-University (LMU), Munich, Germany.

Received: 3 April 2019 Accepted: 15 October 2019

References

1. Singh S, Hamdy S. Dysphagia in stroke patients. Postgrad Med J. 2006;82:383–91. https://doi.org/10.1136/pgmj.2005.043281 .
2. Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke: incidence, diagnosis, and pulmonary complications. Stroke. 2005;36:2756–63. https://doi.org/10.1161/01.STR.0000190056.76543.eb .
3. Heuschmann PU, Kolominsky-Rabas PL, Misselwitz B, Hermanek P, Leffmann C, Janzen RWC, et al. Predictors of in-hospital mortality and attributable risks of death after ischemic stroke: the German stroke registers study group. Arch Intern Med. 2004;164:1761–8. https://doi.org/10.1001/archinte.164.16.1761.
4. Hajat C, Hajat S, Sharma P. Effects of poststroke pyrexia on stroke outcome: a meta-analysis of studies in patients. Stroke. 2000;31:410–4.
5. Yoo S-H, Kim JS, Kwon SU, Yun S-C, Koh J-Y, Kang D-W. Undernutrition as a predictor of poor clinical outcomes in acute ischemic stroke patients. Arch Neurol. 2008;65:39–43. https://doi.org/10.1001/archneurol.2007.12 .
6. Finestone HM, Greene-Finestone LS, Wilson ES, Teasell RW. Malnutrition in stroke patients on the rehabilitation service and at follow-up: prevalence and predictors. Arch Phys Med Rehabil. 1995;76:310–6.
7. Bonilha HS, Simpson AN, Ellis C, Mauldin P, Martin-Harris B, Simpson K. The one-year attributable cost of post-stroke dysphagia. Dysphagia. 2014;29:545–52. https://doi.org/10.1007/s00455-014-9543-8 .
8. Smithard DG, Smeeton NC, Wolfe CDA. Long-term outcome after stroke: does dysphagia matter? Age Ageing. 2007;36:90–4. https://doi.org/10.1093/ageing/afl149 .
9. Warnecke T, Ritter MA, Kroger B, Oelenberg S, Teismann I, Heuschmann PU, et al. Fiberoptic endoscopic dysphagia severity scale predicts outcome after acute stroke. Cerebrovasc Dis. 2009;28:283–9. https://doi.org/10.1159/000228711 .
10. Arnold M, Liesirova K, Broeg-Morvay A, Meisterernst J, Schlager M, Mono M-L, et al. Dysphagia in acute stroke: incidence. Burden and Impact on Clinical Outcome PLoS One. 2016;11:e0148424. https://doi.org/10.1371/journal.pone.0148424 .
11. Gonzalez-Fernandez M, Brodsky MB, Palmer JB. Poststroke communication disorders and dysphagia. Phys Med Rehabil Clin N Am. 2015;26:657–70. https://doi.org/10.1016/j.pmr.2015.06.005 .
12. Braun T, Juenemann M, Viard M, Meyer M, Fuest S, Reuter I, et al. What is the value of fibre-endoscopic evaluation of swallowing (FEES) in neurological patients? A cross-sectional hospital-based registry study BMJ Open. 2018;8:e019016. https://doi.org/10.1136/bmjopen-2017-019016 .
13. Trapl M, Enderle P, Nowotny M, Teuschl Y, Matz K, Dachenhausen A, Brainin M. Dysphagia bedside screening for acute-stroke patients: the Gugging swallowing screen. Stroke. 2007;38:2948–52. https://doi.org/10.1161/STROKEAHA.107.483933 .
14. Langmore SE. Endoscopic evaluation and treatment of swallowing disorders. New York, NY: Thieme; 2001.
15. Langmore SE, Schatz K, Olsen N. Fiberoptic endoscopic examination of swallowing safety: a new procedure. Dysphagia. 1988;2:216–9.
16. Rosenbek JC, Robbins JA, Roecker EB, Coyle JL, Wood JL. A penetration-aspiration scale. Dysphagia. 1996;11:93–8.
17. Daniels SK, Huckabee ML. Dysphagia following stroke. San Diego, CA: Plural Publishing Inc; 2014.
18. Crary MA, Mann GDC, Groher ME. Initial psychometric assessment of a functional oral intake scale for dysphagia in stroke patients. Arch Phys Med Rehabil. 2005;86:1516–20. https://doi.org/10.1016/j.apmr.2004.11.049 .
19. Dziewas R, Warnecke T, Olenberg S, Teismann I, Zimmermann J, Kramer C, et al. Towards a basic endoscopic assessment of swallowing in acute stroke - development and evaluation of a simple dysphagia score. Cerebrovasc Dis. 2008;26:41–7. https://doi.org/10.1159/000135652 .
20. Lindner-Pfleghar B, Neugebauer H, Stosser S, Kassubek J, Ludolph A, Dziewas R, et al. Dysphagiemanagement beim akuten Schlaganfall: Eine prospektive Studie zur Uberprufung der geltenden Empfehlungen. Nervenarzt. 2017;88:173–9. https://doi.org/10.1007/s00115-016-0271-1 .
21. Dziewas R, Matthias auf dem Brinke , Birkmann U, Bräuer G, Busch K, Cerra F, et al. Safety and clinical impact of FEES – results of the FEES-registry. Neurol. Res. Pract. 2019;1:2756. doi:https://doi.org/10.1186/s42466-019-0021-5 .
22. Steele CM, Alsanei WA, Ayanikalath S, Barbon CEA, Chen J, Cichero JAY, et al. The influence of food texture and liquid consistency modification on swallowing physiology and function: a systematic review. Dysphagia. 2015;30:2–26. https://doi.org/10.1007/s00455-014-9578-x .
23. Teismann IK, Dziewas R, Steinstraeter O, Pantev C. Time-dependent hemispheric shift of the cortical control of volitional swallowing. Hum Brain Mapp. 2009;30:92–100. https://doi.org/10.1002/hbm.20488 .
24. Andre JM, Beis JM, Morin N, Paysant J. Buccal hemineglect. Arch Neurol. 2000;57:1734–41.
25. Parker C, Power M, Hamdy S, Bowen A, Tyrrell P, Thompson DG. Awareness of dysphagia by patients following stroke predicts swallowing performance. Dysphagia. 2004;19:28–35. https://doi.org/10.1007/s00455-003-0032-8 .
26. Bax L, McFarlane M, Green E, Miles A. Speech-language pathologist-led fiberoptic endoscopic evaluation of swallowing: functional outcomes for patients after stroke. J Stroke Cerebrovasc Dis. 2014;23:e195–200. https://doi.org/10.1016/j.jstrokecerebrovasdis.2013.09.031 .
27. Hannawi Y, Hannawi B, Rao CPV, Suarez JI, Bershad EM. Stroke-associated pneumonia: major advances and obstacles. Cerebrovasc Dis. 2013;35:430–43. https://doi.org/10.1159/000350199 .

Publisher's Note

5.6. Flexibel endoscopic evaluation of swallowing (FEES) to determine neurological intensive care patient's oral diet[212]

Tobias Braun*, Martin Jünemann*, Maxime Viard, Marco Meyer, Iris Reuter, Stefan Mausbach, Johanna M Doerr, Ingo Schirotzek, Mario Prosiegel, Patrick Schramm, Manfred Kaps, Christian Tanislav

*contributed equally

Auch bei Patienten auf einer neurologischen Intensivstation sind Schluckstörung häufig und gehen ebenfalls mit einer erhöhten Mortalität und Morbidität einher. Da es nur wenige Daten über einen Nutzen der FEES-Untersuchung auf neurologischen Intensivstationen gibt, sahen wir die Notwendigkeit die Untersuchung auf unserer neurologischen Intensivstation weiter zu untersuchen.

Die FEES-Untersuchung wurde bei Intensivpatienten mit dem Verdacht auf eine Schluckstörung durchgeführt. Es erfolgte eine Analyse bezüglich epidemiologischer Basisparameter, Ausmaß der Behinderung, Diagnose einer Pneumonie, Dauer des stationären Aufenthalts, Notwendigkeit maschineller Beatmung oder einer Tracheotomie.

Insgesamt konnten 125 Patienten, bei denen eine Dysphagie vermutet wurde, für die Analyse eingeschlossen werden. Der Großteil der Patienten wurde aufgrund eines akuten Schlaganfalls behandelt (64,8%). Mit Hilfe der FEES wurde bei 72% der Patienten eine oropharyngeale Dysphagie festgestellt. Eine Anpassung der Ernährung wurde in 64% der Fälle nach der FEES empfohlen. Das klinische Outcome bei Entlassung war bei Schlaganfallpatienten, bei denen mittels FEES eine Dysphagie festgestellt wurde, schlechter im Vergleich zu Patienten ohne Dysphagie (mRS median 5 [IQR 3,25-5] vs. median 4 [3-4]; p=0,009). Patienten mit oraler Nahrungskarenz nach der FEES mussten häufiger intubiert werden (67,2% vs. 42,2%; p=0,007), häufiger tracheotomiert werden (19,7% vs. 6,3%; p=0,032) und hatten eine höhere Mortalität (21,3% vs. 1,6%; p<0,001) im Vergleich zu Patienten, die zumindest mit geringen Mengen oder mehr oral ernährt wurden.

Da auch auf der Intensivstation eine Dysphagie nur selten klinisch korrekt eingeschätzt wurde, könnte der niederschwellige Einsatz der FEES bei neurologischen Intensivpatienten helfen, die Ernährung der Patienten adäquat, also entsprechend ihrer Fähigkeiten, anzupassen. Dies könnte zu einer geringeren Mortalität und Morbidität beitragen.

International Journal of Speech-Language Pathology, 2020; Early Online: 1–9

Flexible endoscopic evaluation of swallowing (FEES) to determine neurological intensive care patients' oral diet

TOBIAS BRAUN[1]*, MARTIN JUENEMANN[1]*, MAXIME VIARD[1], MARCO MEYER[1,2], IRIS REUTER[1], STEFAN MAUSBACH[1], JOHANNA M DOERR[1], INGO SCHIROTZEK[1], MARIO PROSIEGEL[3], PATRICK SCHRAMM[4], MANFRED KAPS[1] & CHRISTIAN TANISLAV[1,2]

[1]*Department of Neurology, University Hospital Giessen and Marburg, Giessen, Germany,* [2]*Department of Neurology/Geriatrics, Diakonie Klinikum Jung-Stilling, Siegen, Germany,* [3]*Faculty of Languages and Literatures, Department I, Ludwig-Maximilians-University (LMU), Munich, Germany, and* [4]*Department of Anaesthesiology, University Hospital Mainz, Mainz, Germany*

Abstract

Purpose: Dysphagia is common in critically ill neurological patients and is associated with a high mortality and morbidity. Data on the usefulness of flexible endoscopic examination of swallowing (FEES) in neurological intensive care unit (ICU) patients are lacking, raising the need for evaluation.
Method: FEES was performed in neurological intensive care patients suspected of dysphagia. We correlated findings with baseline data, disability status, pneumonia and duration of hospitalisation, as well as a need for mechanical ventilation or tracheotomy.
Result: This analysis consisted of 125 patients with suspected dysphagia. Most of the patients (81; 64,8%) suffered from acute stroke. Dysphagia was diagnosed using FEES in 90 patients (72%). FEES results led to dietary modifications in 80 patients (64%). The outcome at discharge was worse in dysphagic stroke patients diagnosed by FEES as compared to non-dysphagic stroke patients ($p = 0.009$). Patients without oral diet had higher need for intubation ($p = 0.007$), tracheotomy ($p = 0.032$) and higher mortality ($p < 0.001$) in comparison to patients with at least small amounts of oral intake.
Conclusion: As the clinical assessment of the patients often classified the dysphagia incorrectly, the broad use of FEES in ICU patients might help to adequately adjust patients' oral diet. This knowledge might contribute to lower mortality and morbidity.

Abbreviations: BSE: bedside screening examination; CSE: clinical swallowing examination; FEDSS: fibre endoscopic dysphagia severity scale; FEES: flexible endoscopic examination of swallowing; FOIS: functional oral intake scale; GUSS: Gugging Swallowing Screen; ICU: intensive care unit; IQR: interquartile range; mRS: modified Rankin-scale; NIHSS: National Institute of Health stroke scale; NPO: nil per os (no oral intake); PEG: percutaneous endoscopic gastrostomy tube; SLP: speech-language pathologist.

Keywords: *Intensive care, FEES; dysphagia, aspiration, deglutition disorders*

Introduction

Dysphagia, which is common amongst critically ill patients, is associated with increased mortality and morbidity (Komiya et al., 2013). It predisposes patients to aspiration pneumonia, malnutrition and exsiccosis (Macht, Wimbish, Bodine, & Moss, 2013). Dysphagia therefore determines the immediate prognosis and quality of life of ill patients and is also associated with high healthcare costs (Altman, Yu, & Schaefer, 2010). The frequency of dysphagia in all critically ill patients varies between 15 and 70% (Barker, Martino, Reichardt, Hickey, & Ralph-Edwards, 2009; El Solh, Okada, Bhat, & Pietrantoni, 2003; Macht et al., 2011; Prosiegel et al., 2012). Mechanisms for dysphagia in critically ill patients include anatomical changes after intubation or tracheotomy, muscular weakness due to neuromyopathy (up to 75% of patients with mechanical

Correspondence: Braun Tobias, Department of Neurology, Justus Liebig University, Klinikstrasse 33, Giessen, 35392, Germany. Tel.: +49/64198545372; Fax: +49/6419945418. Email: tobias.braun@neuro.med.uni-giessen.de
*Both authors contributed equally to this work.
Trial registration: Clinicaltrials.gov, NCT03753841. Registered 16th November 2018 – retrospectively registered, https://clinicaltrials.gov/ct2/show/NCT03753841.

ISSN 1754-9507 print/ISSN 1754-9515 online
Published by Taylor & Francis
DOI: 10.1080/17549507.2020.1744727

ventilation for longer than 3 weeks develop critical-illness-polyneuropathy and -myopathy (Puthucheary, Harridge, & Hart, 2010)), decreased sensation in swallowing-relevant areas, changes in the patient's level of consciousness, gastroesophageal reflux and desynchronisation of breathing and swallowing (Macht et al., 2013).

In neurological intensive care unit (ICU) patients, dysphagia is probably even more prevalent, as those patients suffer from damage to regions that are important for swallowing, such as the central pattern generators for swallowing in the medullary brainstem or the supramedullary network that controls the swallowing function (Langmore, 1996). Amongst neurological diseases, dysphagia is very common. It is a frequent cause of death in stroke, multiple sclerosis, neurodegenerative disorders (amyotrophic lateral sclerosis, Parkinson's disease, dementia syndromes) or neuromuscular disorders (Guillain-Barré syndrome ; Cabre et al., 2010; Chen & Garrett, 2005; Heuschmann et al., 2004; Mamolar Andres et al., 2017; Prosiegel, Schelling, & Wagner-Sonntag, 2004). Up to 80% of stroke patients suffer from dysphagia; aspiration pneumonia due to dysphagia is the leading cause of death in stroke (Martino et al., 2005). When severely affected, those patients are usually treated on an ICU.

The diagnostic tools for dysphagia are either a physical examination (clinical swallowing examination; CSE) by a physician or speech-language pathologist (SLP), videofluoroscopy or flexible endoscopic examination of swallowing endoscopy (FEES). Strengths of FEES over videofluoroscopy for patients in the ICU are portability of FEES to be completed at bedside and ability to detect pharyngeal and laryngeal accumulation of secretions as well as aspiration of secretions. Accumulations of saliva in the laryngeal aditus are predictive for aspiration (Murray, Langmore, Ginsberg, & Dostie, 1996). Therefore, FEES represents a valuable tool for the examination of neurological patients in ICU-patients.

Some studies have already suggested the use of FEES on the ICU, but they did not focus on neurological patients and did not compare FEES to clinical assessment or report outcome related parameters, such as pneumonia rate (El Solh et al., 2003; Hafner, Neuhuber, Hirtenfelder, Schmedler, & Eckel, 2008).

To assess the value of FEES in neurological intensive care patients, we evaluated data from all patients in our department who received FEES during the course of 28 months. The study was designed as an observational study. We analysed the data for differences from FEES to clinical assessment and resulting change in oral diet, for differences in patients with and without dysphagia in regard to pneumonia, mortality, ventilation and length of stay in hospital and other outcome related parameters. Due to the fact that most of the patients on a neurological ICU suffer from stroke, we performed a subgroup analysis for this cohort. We also did the same analyses for patients with no oral feeding as compared to patients with at least small amounts of food or fluids. Patients without oral feeding are prone to malnutrition, even when treated in a hospital receiving parenteral or enteral feeding via nasogastric feeding tube (Butterworth, 1994; "Disease-related malnutrition and enteral nutrition therapy: a significant problem with a cost-effective solution," 2010; Souza, Sturion, & Faintuch, 2015). Literature suggests a worse outcome in patients with no oral feeding, as they are predisposed to malnutrition (Newman et al., 2001; Schumann et al., 2012). Corresponding data from neurological ICU patients could not be identified at the time of data acquisition.

Method

The study was undertaken in a large German university hospital. In patients diagnosed with neurological disorders other than stroke, FEES was executed when there were pathological findings (facial palsy, abnormal gag reflex or volitional cough, dysarthria/dysphonia, etc.) in the CSE conducted by an SLP. The indication for CSE was a clinical suspicion of dysphagia (i.e. recurring pneumonia) or clinical signs of dysphagia (e.g. a wet voice and/or coughing when drinking, etc.), reported by nurses, SLPs or the treating physicians. The patients with signs of dysphagia were discussed among the "dysphagia experts" of our department (SLPs or physicians with more than 5 years of expertise in FEES and dysphagia) and indication for FEES was confirmed. The Gugging Swallowing Screen (GUSS) was used as a bedside screening examination (BSE) for stroke-associated dysphagia. The GUSS consists of four subtests, testing vigilance, the abilities to cough and swallow saliva (subtest 1) and different consistencies of food (liquid, semi-solid, solid; subtests 2–4). In each subtest, a maximum of 5 points can be reached. The sum of points determines the patient's severity of dysphagia and based on the score, different diet recommendations are given (Trapl et al., 2007).

In stroke patients, an oral diet prior to FEES was chosen according to the instructions yielded by the GUSS. For diagnoses different from stroke, no scales exist. Therefore, clinical judgement by SLP or attending physician was necessary to determine the oral diet in non-stroke patients. As there were only 2 SLPs in our department, low variation in the clinical judgement was to be expected. For quality control reasons, the findings gathered in examinations were systematically documented. All FEES procedures were performed or supervised and rated in a standardised manner by experienced physicians (see below). The physicians had at least 4 years of experience in FEES. The time from CSE or GUSS to FEES was 72 h in the majority of patients.

Study population

All patients treated within the ICU in our department who received FEES from January 2014 to September 2016 were considered for the analysis. Data documented in the database included gender, age, diagnosis, the presence of brain lesions (as in new or old ischaemic stroke, intracerebral bleeding, tumour, cerebral atrophy, etc.), functional outcome in stroke patients (National Institute of Health Stroke Scale [NIHSS]/modified Rankin scale [mRS] on admission and at discharge), need for intubation and re-intubation, tracheotomy, overall time required for mechanical ventilation, time of mechanical ventilation lasting >24 h, length of hospital stay, occurrence of pneumonia, mortality, a percutaneous endoscopic gastrostomy (PEG) procedure, presence of dysphagia and type of oral intake (before and after FEES). With respect to data acquisition and scientific analyses, an ethical approval was obtained from the local ethical committee (protocol number 208/16).

FEES – flexible endoscopic examination of swallowing

FEES is a videoendoscopic nasolaryngeal swallowing study that was performed according to the standardised FEES® protocol according to Langmore [12]: after applying decongestants (Xylometazoline) and local anaesthesia of the nasal duct using 2% Lidocaine-gel, a small endoscope (about 4 mm in diameter) was introduced through the inferior nasal meatus and the epipharynx in the mesopharynx. In the first step of the procedure, anatomical changes, management of saliva and movements of swallowing related structures were assessed.

We then tested different consistencies of food and liquids. Penetration, aspiration, localisation and amount of residues, as well as patients' reactions (such as coughing), were visualised and documented. By definition, penetration is the entry of any material into the airway (above the level of the vocal folds) and aspiration means the entry of any material below the level of the vocal folds. The consistencies we used were pudding-thick consistency (thickened water), normal water and solid food (Langmore, Schatz, & Olsen, 1988). For every consistency, we first used a teaspoon, then a tablespoon and in case of water, the patient was asked to take a normal swallow from a cup. Each test was applied three times. For better visualisation, the consistencies were dyed blue, using food colour. The consistence was rated as unsafe in case of entering of the airway to the level of the vocal folds without ejection from the airway or any aspiration (Score 5–8 on Rosenbek's Penetration-Aspiration-Scale, Rosenbek, Robbins, Roecker, Coyle, & Wood, 1996). If one of the consistencies appeared unsafe, this finding was implemented in a corresponding dietary restriction A consistency was rated unsafe, if a patient failed two out of the three times tested or a large amount of bolus entered the airway in a single time.

Based on the findings in FEES, the appropriate oral diet was chosen for the patients.

Outcome measurement

Oral intake and the degree of dysphagia severity were measured by using the functional oral intake scale (FOIS) and the Fiberoptic Endoscopic Dysphagia Severity Score (FEDSS), respectively.

FOIS is a seven-tiered scale ranging from 1 = no oral intake at all (NPO = nil per os) to 7 = full oral intake without restrictions (Supplementary Appendix 1; Crary, Mann, & Groher, 2005). Scores 2–3 is a mixture of oral and non-oral intake and score 4–6 is total oral diet with dietary restrictions. A reduction in dietary restrictions was defined as a positive change on the FOIS, whereas restriction of the oral diet was defined as a negative change.

In order to define the overall severity of dysphagia, we used the FEDSS scale developed by Dziewas et al. (2008). The FEDSS is a six-tiered scale originally designed for stroke patients (Supplementary Appendix 2) and is based on FEES findings. All parameters are recorded in a standardised way.

Statistical analysis

The following parameters were correlated with baseline data and dependent factors: dysphagia as defined by a FEDSS score of ≥2 and oral intake status as calculated by the FOIS after FEES.

Outcome variables were either dummy coded (gender, presence of a structural brain lesion, need for intubation, re-intubation, tracheotomy, long-term ventilation [≥24 h], diagnosis of pneumonia, death, PEG-tube at discharge, a change of the oral diet regime, restriction of oral diet, reduction of restrictions of oral diet and start of NPO) or depending on ordinal scales (age, time of mechanical ventilation in hours, length of stay in hospital, mRS and NIHSS) which, due to the mostly severely impaired nature of the included patients, resulted in non-normally distributed data.

We therefore calculated absolute and relative frequencies based on cross tables. For group comparisons of dummy coded variables (0/1), the Chi Square test was used. We further employed the non-parametric Mann–Whitney *U*-test for group comparisons of the remaining variables. All statistical analyses were performed with the SPSS, release version 22.0 (©SPSS, Inc., IBM Company, 2015, Chicago, IL).

Result

Patients' characteristics

In 125 patients, 144 FEES were performed. In order to prevent distortion of data, only the results of the first examination were included in the analysis of patients who received more than one FEES. In 11

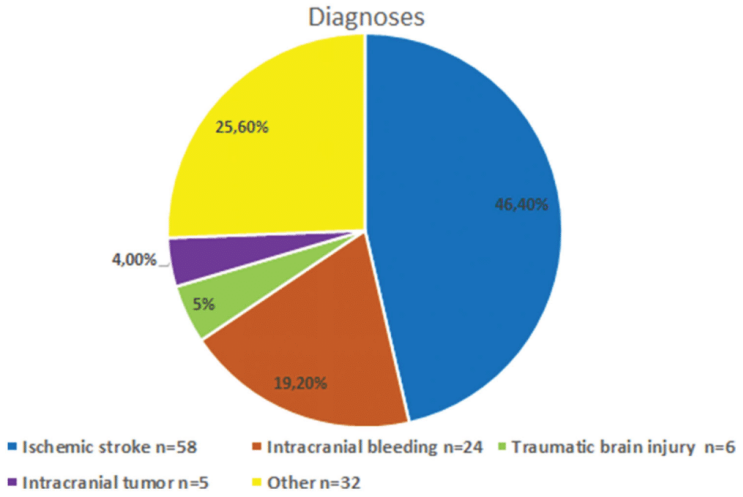

Figure 1. Disease entities detected in patients in percentage and absolute.

patients (8.8%), the procedure was repeated at least once. Among the patients, 80 were male (64%), the median age was 71 years (interquartile range [IQR] 59–77 years). Further, the largest subgroup of patients (58; 46,4%) suffered a stroke. The different disease entities detected in our patients are summarised in Figure 1. The group classified as "other" includes patients with heterogeneous diagnoses (motoneuron disease, movement disorders, epileptic seizures, Guillain-Barré syndrome, etc.). Amongst all patients, 105 (84%) showed a brain lesion detected in cerebral computed tomography or magnetic resonance imaging (5 tumours, 58 new ischaemic lesions, 30 primary haemorrhages, 12 lesions of other kind – old ischaemic lesions, unspecific white matter lesions, cortical atrophy, etc.), 68 (54.8%) were intubated at any given time, 18 (14.4%) had to be reintubated, and 16 (12.8%) underwent percutaneous dilatative tracheotomy. The total time of mechanical ventilation was a median of 170 h (IQR 23–256 h). When ventilation was required for longer than 24 h, which was necessary in 53 patients (42.4%), the time of mechanical ventilation was a median of 295 h (IQR 113.25–605.5 h). In addition, 59 patients (47.2%) were diagnosed with pneumonia at any given time during hospitalisation, 14 patients (11.2%) died during hospitalisation. Initially, after GUSS or CSE, the initial diet recommendation in 78 patients (62.4%) was no oral intake (NPO), in 13 patients (10.4%) a mixture of oral and non-oral intake, in 22 patients (17.6%) oral intake with dietary restrictions and in 12 patients (9.6%) full oral intake. Further, 74 patients (59.2%) had a nasogastric feeding tube and 4 patients (3.2%) had a PEG (percutaneous endoscopic gastrostomy) tube prior to FEES. The median length of stay in hospital was 24 days (IQR 16–36.5 days).

FEES examination

The median FEDSS of the entire study population was 4 (IQR 1–6). Using FEES, we diagnosed dysphagia (FEDSS 2–6) in 72% of the patients (90 patients).

A diet modification of the initial diet recommendation after CSE or GUSS was necessary in 80 patients (64%) with a reduction in diet restrictions in 53 patients (66.3%) and restriction of the diet in 27 patients (33.7%). The diet was modified in 52 (64.2%) of the stroke patients receiving the GUSS and in 28 (63.6%) of the non-stroke patients with a CSE ($p > 0.99$). Forty patients (32%), with NPO or the mixture diet with oral and non-oral intake, had a full oral intake with or without diet restrictions after FEES. The nasogastric feeding tube was removed in 27 patients (21.6%). In 8 patients (6.4%) with full oral intake, the procedure unveiled a critical dysphagia and, as a result, the diet strategy was re-evaluated. Amongst these patients, 2 had full oral intake with dietary restrictions and 6 had NPO after FEES. Changes in the oral diet based on FEES can be seen in Figure 2. Among the 80 patients, whose diet strategy had to be revised due to differing results from screening and CSE, FEES detected silent aspiration in 23 patients (28.8%).

The results are summarised in Table I.

No side effects from the FEES procedure (i.e. laryngospasm, syncope or non-self-limiting epistaxis) occurred; however, 1 patient (0.8%) suffered mild epistaxis after FEES.

Stroke subgroup

Among the stroke patients, median age was 71 (IQR 59–77). Fifty-eight patients (71.6%) suffered an

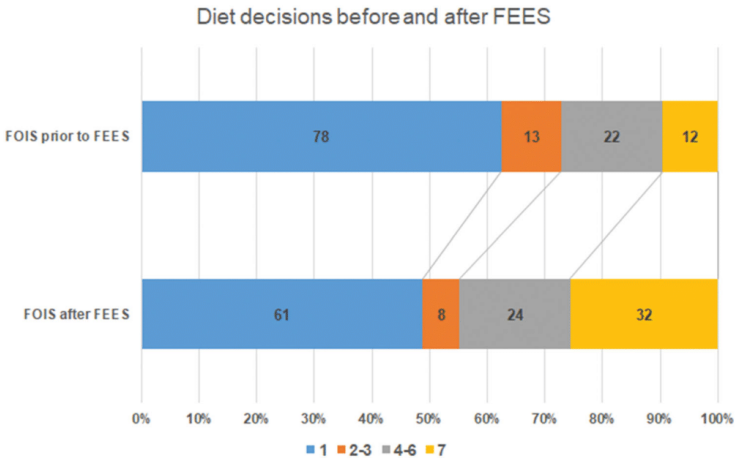

Figure 2. Decisions of patients diet before and after FEES (flexible endoscopic examination of swallowing) in patients in regard to. The number in the bars is the number of patients.

Table I. Baseline characteristics in patients with and without dysphagia.

	Non-dysphagic patients (FEDSS = 1; $n = 35$)	Dysphagic patients (FEDSS ≥2; $n = 90$)	Chi-squared value (Chi-squared-test)	Effect size (Cramer's V)
Gender (number of male patients)	22 (62.9%)	58 (64.4%)	0.028	0.015
Brain lesion	28 (80%)	77 (85.6%)	0.579	0.068
Intubation	15 (42.9%)	53 (58.9%)	2.611	0.145
Re-Intubation	2 (14.3%)	16 (17.8%)	1.511	0.151
Tracheotomy	3 (8.6%)	13 (14.4%)	0.779	0.079
Long-term ventilation necessary (≥24 h)	11 (31.4%)	42 (46.7)	2.396	0.122
Pneumonia	14 (40%)	45 (50%)	1.011	0.09
Death	1 (2.9%)	13 (14.4%)	3.402	0.165
PEG at discharge	6 (17.1%)	23 (25.6%)	1.001	0.089
Change in oral diet	31 (88.6%)	49 (54.4%)	12.738***	0.319
Restriction	0	27 (30%)	13.393***	0.327
NPO started	0	22 (24.4%)	10.383**	0.288
Reduction of restrictions	31 (88.6%)	22 (24.4%)	42.432***	0.583

	Non-dysphagic patients (FEDSS = 1; $n = 35$)	Dysphagic patients (FEDSS ≥2; $n = 90$)	U-value (Mann–Whitney *U*-test)	Effect size (η^2)
Age median (IQR)	69 (59–76)	71 (59–77)	1460	0.003
Time of mechanical ventilation in hours (median, IQR)	156.5 (30.25–326.25)	170 (22.5–537.5)	361.5	0.004
Time of mechanical long-term ventilation in hours (≥24 h; media, IQR)	179 (111–327)	346 (114–673)	169	0.045
Length of stay in hospital in days (median, IQR)	22 (16–30)	24.5 (16–39.25)	1338	0.019

*$p < 0.05$,
**$p < 0.01$,
***$p < 0.001$.
Effect sizes: Cramer's V: 0.1 = small, 0.3 = medium, >0.5 = large, η^2: 0.01 = small, 0.06 = medium, >0.14 = large. FEDSS: Fiberendoscopic dysphagia severity scale; FEES: flexible endoscopic examination of swallowing; IQR: interquartile range; PEG: percutaneous endoscopic gastrostomy; NPO: nil per os.

ischaemic stroke and 23 (28.4%) an intracranial bleeding. Thirty-eight patients (46.9%) were intubated with a need for re-intubation after extubation in 7 (8.6%) of the stroke patients. The total time of mechanical ventilation was a median of 88 h (IQR 23–479 h). When ventilation was required for longer than 24 h, which was necessary in 29 patients (35.8%), the time of mechanical ventilation was a median of 193 h (IQR 67.5 – 496.5 h). Eight patients underwent tracheotomy (9.9%). Forty-one patients were diagnosed with pneumonia (50.6%), 8 stroke patients (9.9%) died. Twenty patients (24.7%) were discharged with a PEG tube. On admission, the median NIHSS was 13 (IQR 6.25–19.75) and the median mRS was 5 (IQR 4–5). At discharge, the median NIHSS was 7 (IQR 4–12) and the median mRS was 4 (IQR 3–5). The median length of stay in hospital in the stroke patient subgroup was 23 days (IQR 14.5–32.5 days). The diet regime according to the GUSS or CSE was revised after FEES in 52 patients (64.2%), with a restriction of oral diet in 18 patients (22.2%) and a reduction of dietary restrictions in 34 patients (42%). In 15 patients (18.5%), NPO was ordered.

Table II. Baseline characteristics in patients with and without dysphagia in the subgroup of patients with stroke.

	Non-dysphagic patients (FEDSS = 1; $n = 24$)	Dysphagic patients (FEDSS ≥ 2; $n = 57$)	Chi-squared value (Chi-squared-test)	Effect size (Cramer's V)
Ischaemic stroke	42 (72.4%)	16 (27.6%)		
Intracranial bleeding	8 (34.8)	15 (65.2%)		
Gender (number of male patients)	16 (66.7%)	38 (66.7%)	<0.001	<0.001
Intubation	7 (29.2%)	31 (54.4%)	4.313*	0.231
Re-Intubation	0	7 (12.3%)	2.014	0.233
Tracheotomy	1 (4.2%)	7 (12.3%)	1.249	0.124
Long-term ventilation necessary (≥24 h)	4 (16.7%)	25 (43.9%)	5.434*	0.259
Pneumonia	10 (41.7%)	31 (54.4%)	1.093	0.116
Death	0	8 (14%)	3.783	0.215
PEG at discharge	4 (16.7%)	16 (28.1%)	1.181	0.121
Change in oral diet	22 (91.7%)	30 (52.6%)	11.196**	0.372
Restriction	0	18 (31.6%)	9.744**	0.347
NPO started	0	15 (26.3%)	7.751**	0.309
Reduction of restrictions	22 (91.7%)	12 (21.1%)	34.576***	0.653

	Non-dysphagic patients (FEDSS = 1; $n = 24$)	Dysphagic patients (FEDSS ≥ 2; $n = 57$)	U-value (Mann–Whitney *U*-test)	Effect size (η^2)
Age (median, IQR)	68.5 (56.75–79)	73 (60–73)	612.5	0.007
Stroke severity on admission				
NIHSS on admission; median (IQR)	9 (6–13)	14 (8.25–20.75)	445.5*	0.072
mRS on admission; median (IQR)	4 (3–5)	5 (4–5)	430**	0.108
Stroke severity at discharge				
NIHSS at discharge; median (IQR)	5.5 (3–10)	9 (4–14)	447.5	0.038
mRS at discharge; median (IQR)	4 (3–4)	5 (3.25–5)	432.5**	0.086
Time of mechanical ventilation in hours (media, IQR)	52.5 (21.75–165)	114 (24–487.5)	74.5	0.024
Time of mechanical long-term ventilation in hours (≥24 h; media, IQR)	91.5 (42.75–273)	241 (71–521)	30	0.057
Length of stay in hospital in days (median, IQR)	18 (14.25–27.75)	23 (14.5–34)	583	0.014

*$p < 0.05$,
**$p < 0.01$,
***$p < 0.001$.
Effect sizes: Cramer's V: 0.1 = small, 0.3 = medium, >0.5 = large, η^2: 0.01 = small, 0.06 = medium, >0.14 = large. FEDSS: Fiberendoscopic dysphagia severity scale; FEES: flexible endoscopic examination of swallowing; IQR: interquartile range; NIHSS: National Institute of Health stroke scale; mRS: modified Rankin Scale; PEG: percutaneous endoscopic gastrostomy; NPO: nil per os; e: number of patients.

In the stroke patient subgroup, 57 patients (70.3%) were diagnosed with dysphagia. The median FEDSS in the stroke subgroup was 4 (IQR 1–6). In dysphagic stroke patients, NIHSS and mRS on admission and at discharge were higher than in non-dysphagic stroke patients (Admission: NIHSS 14 vs. 9, $p = 0.017$; mRS 5 vs. 4, $p = 0.004$; Discharge: NIHSS 9 vs. 5.5, $p = 0.098$; mRS 5 vs. 4, $p = 0.009$). Dysphagic stroke patients had a higher need for intubation (54.4% vs 29.2%, $p = 0.038$) and a higher need for long-term ventilation (43.9% vs. 16.7%, $p = 0.02$) compared to non-dysphagic stroke patients. There was a trend towards a higher mortality in dysphagic stroke patients as compared to stroke patients without dysphagia (14% vs. 0%; $p = 0.053$).

The side of the ischaemic lesion or bleeding and the involved vascular territory had no impact on the presence of dysphagia in stroke patients (data not shown). The results in the subgroup of stroke patients are summarised in Table II.

Differences in patients with NPO and oral diet after FEES

Compared to patients with at least small amounts of oral intake (FOIS ≥2), patients for whom NPO was ordered had a higher need for intubation (67.2% vs. 42.2%, $p = 0.005$), tracheotomy (19.7% vs. 6.3%, $p = 0.025$) and higher mortality rates (21.3% vs. 1.6%, $p < 0.001$) and a longer time of mechanical ventilation, when ventilation was necessary for more than 24 h (179 h vs. 467 h; $p = 0.033$). Stroke patients with NPO had a worse functional outcome at discharge (median mRS 5 vs. 4, $p < 0.001$) as compared to patients with at least small amount of oral intake. The results are summarised in Table III.

Discussion

In 72% of our neurological intensive care patients, we detected dysphagia using FEES.

Amongst our patient pool, 64% needed a modification of their diet strategy that was originally recommended based on GUSS-screening for stroke patients or CSE alone in non-stroke-patients – an alarming number, as only roughly 1/3 of our patients had an adequate diet based on their swallowing abilities in FEES. In about 1/3 of the patients, FEES detected silent aspiration. This outcome demonstrates the inability to detect silent aspiration using screening tools or clinical assessment in neurological ICU-patients. Martin-Harris, Logemann, McMahon, Schleicher, and Sandidge (2000) showed that diet modifications determined by a CSE other than those for purely oral stage dysphagia are not always accurate and tend to be more restrictive than

Table III. Differences in patients with oral diet or NPO after FEES.

	Oral diet after FEES (FOIS > 2; $n = 64$)	NPO after FEES (FOIS = 1; $n = 61$)	Chi-squared value (Chi-squared-test)	Effect size (Cramer's V)
Gender (number of male patients)	39 (60.9%)	41 (67.2%)	0.534	0.065
Brain lesion	50 (78.1%)	55 (90.2%)	3.368	0.164
Intubation	27 (42.2%)	41 (67.2%)	7.885**	0.251
Re-Intubation	4 (6.3%)	14 (23%)	2.578	0.198
Tracheotomy	4 (6.3%)	12 (19.7%)	5.041*	0.201
Long-term ventilation necessary (≥24 h)	19 (29.7%)	34 (55.7%)	8.678**	0.263
Pneumonia	27 (42.2%)	32 (52.5%)	1.322	0.103
Death	1 (1.6%)	13 (21.3%)	12.248***	0.313
PEG at discharge	11 (17.2%)	18 (29.5%)	2.661	0.146

	Oral diet after FEES (FOIS > 2; $n = 64$)	NPO after FEES (FOIS = 1; $n = 61$)	U-value (Mann–Whitney *U*-test)	Effect size (η^2)
Age median (IQR)	70 (59.25–76)	72 (58.5–77.5)	1824.5	0.003
Stroke severity on admission (in stroke patients)				
NIHSS on admission; median (mean, IQR)	10 (6–19)	14 (7–20)	676.5	0.018
mRS on admission; median (mean, IQR)	5 (4–5)	5 (4–5)	653.5	0.033
Stroke severity at discharge (in stroke patients)				
NIHSS at discharge; median (mean, IQR)	7 (3.5–11)	9 (4–15.75)	530.5	0.027
mRS at discharge; median (mean, IQR)	4 (3–4)	5 (4–5)	438.5***	0.163
Time of mechanical ventilation in hours (median, IQR)	111 (22–326)	251.5 (33.25–655.75)	459	0.037
Time of mechanical ventilation in hours without short-term ventilation (≥24 h; median, IQR)	179 (72–491)	467 (119–841)	208*	0.087
Length of stay in hospital in days (median, IQR)	21.5 (16–32)	29 (16.5–42)	1638	0.019

*$p < .05$,
**$p < .01$,
***$p < .001$.
Effect sizes: Cramer's V: 0.1 = small, 0.3 = medium, >0.5 = large, η^2: 0.01 = small, 0.06 = medium, >0.14 = large. FEES: flexible endoscopic examination of swallowing; IQR: interquartile range; NIHSS: National Institute of Health stroke scale; mRS: modified Rankin Scale; PEG: percutaneous endoscopic gastrostomy; NPO: nil per os; FOIS: functional oral intake scale.

necessary. Our data are consistent with other studies demonstrating a reduced sensitivity and specificity when performing non-instrumented dysphagia-diagnostics (Hales, Drinnan, & Wilson, 2008; Lynch et al., 2017; McCullough, Wertz, & Rosenbek, 2001).

Individuals in the subgroup of stroke patients had a worse functional status (higher NIHSS and mRS) on admission and at discharge in comparison to non-dysphagic patients. In stroke patients treated on a neurological ICU, the severity of functional deficits seems to be predictive of dysphagia and vice versa, as a higher NIHSS or mRS was associated with a higher FEDSS (data not shown). As reported by Dziewas et al. (2008), penetration and aspiration could be detected when patients had an NIHSS >3. Three months after stroke, Warnecke et al. (2009) were able to show that the degree of dysphagia is predictive of the functional outcome. Stroke patients with dysphagia also needed long-term ventilation more often as compared to stroke patients with normal swallowing function. This result is in agreement with literature and was to be expected, as dysphagia leads to pneumonia and need for ventilation in stroke and ventilation predisposes patients for dysphagia (Chapman, Morgan, Cadilhac, Purvis, & Andrew, 2018; Macht et al., 2013; Wästfelt, Cao, & Ström, 2018). In stroke patients, the adherence to only the GUSS might have an impact to the accuracy of the decisions of the feeding strategy prior to FEES. Using a thorough CSE in those patients might lead to less modifications of oral diet after FEES in stroke patients. However, there were no statistical differences between the GUSS-screening and the thorough CSE. Therefore, we would not expect major differences from our results. In addition, using GUSS, then CSE followed by FEES does not represent the clinical routine for stroke patients, at least on our intensive care unit . As some group comparisons in the stroke subgroup compare small numbers using the Mann–Whitney *U*-test, these comparisons might be underpowered, which might result in non-detection of differences (type 2 error). However, we are confident in the validity of our main results.

Patients who were put on NPO after FEES had a higher mortality, a higher need for intubation and tracheotomy in comparison to patients who had any kind of oral diet. Critically ill patients tend to be undernutritioned, which predisposes them to musclewasting and might worsen the effects of critical illness – polyneuropathy and – myopathy (Latronico & Guarneri, 2008; Yoo et al., 2008). Those conditions lead to a further decline of the swallowing function, resulting in aspiration and pneumonia (Macht et al., 2013).

Our results underline the necessity for instrumental diagnostics. In our opinion, FEES should be performed at a low threshold, especially in the high-risk-group of neurological intensive care patients. FEES is a safe, fast and reliable tool; we observed no serious side effects in approximately 144 examinations that lasted about 10 minutes each. In our opinion, at least in neurological intensive care unit patients, FEES should be performed early on and

straightaway irrespective of GUSS-screening or CSE. However, a CSE is still necessary to have all information to formulate the pathomechanism of dysphagia.

Some studies have already suggested the use of FEES on the ICU, but those studies did not focus on neurological patients (El Solh et al., 2003; Hafner et al., 2008). The study by Hafner et al. reports on the use of FEES in 553 patients with various diseases entities over a period of 45 months. However, they only reported the presence or absence of dysphagia and diet recommendations after FEES. They did not report pneumonia rate, functional outcome or mortality, which is why our study further strengthens the need for instrumented diagnostics.

Dysphagia had no impact on the pneumonia rate in our patients. Our data might be distorted by severely ill patients, who aspirated preclinically and developed pneumonia during the first days of their treatment, because our study design does not differentiate the time of occurrence of pneumonia. The retrospective nature of our data collection also limits our results. These are the main limitations of the study. However, our study design represents the clinical routine of a pre-selection of patients by using a BSE or CSE followed by an instrumented diagnostic.

It would be interesting to know if using FEES has an impact on outcome, pneumonia rate and other factors. A prospective randomised controlled trial with patients receiving FEES or not would be necessary to demonstrate this effect. As only one third of our patients had the adequate diet, we have ethical concerns regarding the design of such a trial, as we would risk exposing our patients to aspiration and pneumonia.

Conclusion

Compared to a clinical assessment, FEES can be regarded as a superior method to detect penetration and aspiration in neurological intensive care patients, as the majority of patients had not the diet based on their swallowing abilities. In more than a quarter of our patients, silent aspiration was not detected by clinical assessment. This understanding is of utmost importance, as the avoidance of dysphagia-related complications might have an impact on mortality and morbidity in this population. FEES can be performed with a low periprocedural risk. We recommend a broad use of FEES in all neurological intensive care patients to screen for dysphagia, choose a diet based on the patients swallowing abilities and to select the proper therapy for the patients.

Ethics approval and consent to participate

For the data acquisition and the use of findings for scientific analyses, an ethical approval was obtained from the local ethical committee (Justus-Liebig University, protocol number 208/16). The ethical committee waived the need for the patients' consent to participate.

Availability of data and material

The authors declare that the data supporting the findings of this study are available within the article. The data that support the findings of this study are not publicly available due to local medical data protection policies.

Disclosure statement

All authors report that there are no conflicts of interest or competing interests related to the presented manuscript.

Supplementary material

Supplemental data for this article can be accessed at https://doi.org/10.1080/17549507.2020.1744727

References

El Solh, A., Okada, M., Bhat, A., & Pietrantoni, C. (2003). Swallowing disorders post orotracheal intubation in the elderly. *Intensive Care Medicine*, *29*, 1451–1455. doi:10.1007/s00134-003-1870-4

Altman, K.W., Yu, G.P., & Schaefer, S.D. (2010). Consequence of dysphagia in the hospitalized patient: Impact on prognosis and hospital resources. *Archives of Otolaryngology–Head & Neck Surgery*, *136*, 784–789. doi:10.1001/archoto.2010.129

Barker, J., Martino, R., Reichardt, B., Hickey, E.J., & Ralph-Edwards, A. (2009). Incidence and impact of dysphagia in patients receiving prolonged endotracheal intubation after cardiac surgery. *Canadian Journal of Surgery. Journal Canadien De Chirurgie*, *52*, 119–124.

Butterworth, C.E. (1994). The skeleton in the hospital closet. *Nutrition (Burbank, Los Angeles County, Calif.)*, *10*, 442.

Cabre, M., Serra-Prat, M., Palomera, E., Almirall, J., Pallares, R., & Clave, P. (2010). Prevalence and prognostic implications of dysphagia in elderly patients with pneumonia. *Age and Ageing*, *39*, 39–45. doi:10.1093/ageing/afp100

Chapman, C., Morgan, P., Cadilhac, D.A., Purvis, T., & Andrew, N.E. (2018). Risk factors for the development of chest infections in acute stroke: A systematic review. *Topics in Stroke Rehabilitation*, *25*, 445–458. doi:10.1080/10749357.2018.1481567

Chen, A., & Garrett, C.G. (2005). Otolaryngologic presentations of amyotrophic lateralsclerosis. *Otolaryngology–Head and Neck Surgery: Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*, *132*, 500–504. doi:10.1016/j.otohns.2004.09.092

Crary, M.A., Mann, G.D.C., & Groher, M.E. (2005). Initial psychometric assessment of a functional oral intake scale for dysphagia in stroke patients. *Archives of Physical Medicine and Rehabilitation*, *86*, 1516–1520. doi:10.1016/j.apmr.2004.11.049

Disease-related malnutrition and enteral nutrition therapy: A significant problem with a cost-effective solution (2010). *Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition*, *25*(5), 548–554. doi:10.1177/0884533610378524

Dziewas, R., Warnecke, T., Olenberg, S., Teismann, I., Zimmermann, J., Kramer, C., … Schabitz, W.R. (2008). Towards a basic endoscopic assessment of swallowing in acute stroke - development and evaluation of a simple

dysphagia score. *Cerebrovascular Diseases (Basel, Switzerland)*, *26*, 41–47. doi:10.1159/000135652

Hafner, G., Neuhuber, A., Hirtenfelder, S., Schmedler, B., & Eckel, H.E. (2008). Fiberoptic endoscopic evaluation of swallowing in intensive care unit patients. *European Archives of Oto-Rhino-Laryngology*, *265*, 441–446. doi:10.1007/s00405-007-0507-6

Hales, P.A., Drinnan, M.J., & Wilson, J.A. (2008). The added value of fibreoptic endoscopic evaluation of swallowing in tracheostomy weaning. *Clinical Otolaryngology: Official Journal of ENT-UK; Official Journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery*, *33*, 319–324. doi:10.1111/j.1749-4486.2008.01757.x

Heuschmann, P.U., Kolominsky-Rabas, P.L., Misselwitz, B., Hermanek, P., Leffmann, C., Janzen, R.W.C., & Berger, K. (2004). Predictors of in-hospital mortality and attributable risks of death after ischemic stroke: The German Stroke Registers Study Group. *Archives of Internal Medicine*, *164*, 1761–1768. doi:10.1001/archinte.164.16.1761

Komiya, K., Ishii, H., Umeki, K., Mizunoe, S., Okada, F., Johkoh, T., & Kadota, J.I. (2013). Impact of aspiration pneumonia in patients with community-acquired pneumonia and healthcare-associated pneumonia: A multicenter retrospective cohort study. *Respirology (Carlton, Vic.)*, *18*, 514–521. doi:10.1111/resp.12029

Langmore, S.E. (1996). Dysphagia in neurologic patients in the intensive care unit. *Seminars in Neurology*, *16*, 329–340. doi:10.1055/s-2008-1040991

Langmore, S.E., Schatz, K., & Olsen, N. (1988). Fiberoptic endoscopic examination of swallowing safety: A new procedure. *Dysphagia*, *2*, 216–219. doi:10.1007/BF02414429

Latronico, N., & Guarneri, B. (2008). Critical illness myopathy and neuropathy. *Minerva Anestesiologica*, *74*, 319–323.

Lynch, Y.T., Clark, B.J., Macht, M., White, S.D., Taylor, H., Wimbish, T., & Moss, M. (2017). The accuracy of the bedside swallowing evaluation for detecting aspiration in survivors of acute respiratory failure. *Journal of Critical Care*, *39*, 143–148. doi:10.1016/j.jcrc.2017.02.013

Macht, M., Wimbish, T., Bodine, C., & Moss, M. (2013). Icu-acquired swallowing disorders. *Critical Care Medicine*, *41*, 2396–2405. doi:10.1097/CCM.0b013e31829caf33

Macht, M., Wimbish, T., Clark, B.J., Benson, A.B., Burnham, E. L., Williams, A., & Moss, M. (2011). Postextubation dysphagia is persistent and associated with poor outcomes in survivors of critical illness. *Critical Care (London, England)*, *15*, R231. doi:10.1186/cc10472

Mamolar Andres, S., Santamarina Rabanal, M.L., Granda Membiela, C.M., Fernandez Gutierrez, M.J., Sirgo Rodriguez, P., & Alvarez Marcos, C. (2017). Swallowing disorders in Parkinson's disease [Trastornos de la deglucion en la enfermedad de Parkinson]. *Acta Otorrinolaringologica Espanola*, *68*, 15–22. doi:10.1016/j.otorri.2016.02.001

Martin-Harris, B., Logemann, J.A., McMahon, S., Schleicher, M., & Sandidge, J. (2000). Clinical utility of the modified barium swallow. *Dysphagia*, *15*, 136–141. doi:10.1007/s004550010015

Martino, R., Foley, N., Bhogal, S., Diamant, N., Speechley, M., & Teasell, R. (2005). Dysphagia after stroke: Incidence, diagnosis, and pulmonary complications. *Stroke*, *36*, 2756–2763. doi:10.1161/01.STR.0000190056.76543.eb

McCullough, G.H., Wertz, R.T., & Rosenbek, J.C. (2001). Sensitivity and specificity of clinical/bedside examination signs for detecting aspiration in adults subsequent to stroke. *Journal of Communication Disorders*, *34*, 55–72. doi:10.1016/S0021-9924(00)00041-1

Murray, J., Langmore, S.E., Ginsberg, S., & Dostie, A. (1996). The significance of accumulated oropharyngeal secretions and swallowing frequency in predicting aspiration. *Dysphagia*, *11*, 99–103. doi:10.1007/BF00417898

Newman, A.B., Yanez, D., Harris, T., Duxbury, A., Enright, P.L., & Fried, L.P., for the Cardiovascular Study Research Group. (2001). Weight change in old age and its association with mortality. *Journal of the American Geriatrics Society*, *49*, 1309–1318. doi:10.1046/j.1532-5415.2001.49258.x

Prosiegel, M., Riecker, A., Weinert, M., Dziewas, R., Lindner-Pfleghar, B., Stanschus, S., & Warnecke, T. (2012). Dysphagiemanagement in der akuten Schlaganfallphase [Management of dysphagic patients with acute stroke]. *Der Nervenarzt*, *83*, 1590–1599. doi:10.1007/s00115-012-3679-2

Prosiegel, M., Schelling, A., & Wagner-Sonntag, E. (2004). Dysphagia and multiple sclerosis. *International MS Journal*, *11*, 22–31.

Puthucheary, Z., Harridge, S., & Hart, N. (2010). Skeletal muscle dysfunction in critical care: Wasting, weakness, and rehabilitation strategies. *Critical Care Medicine*, *38*, S676–S82. doi:10.1097/CCM.0b013e3181f2458d

Rosenbek, J.C., Robbins, J.A., Roecker, E.B., Coyle, J.L., & Wood, J.L. (1996). A penetration-aspiration scale. *Dysphagia*, *11*, 93–98. doi:10.1007/BF00417897

Schumann, B., Büßelberg, N., Stanschus, S., Willmes, K., Andres, F., & Binkofski, F. (2012). Risikofaktoren für Pneumonie bei akuten Schlaganfallpatienten mit Dysphagie. *Sprache · Stimme · Gehör*, *36*, e20–e21. doi:10.1055/s-0032-1304897

Souza, T.T., Sturion, C.J., & Faintuch, J. (2015). Is the skeleton still in the hospital closet? A review of hospital malnutrition emphasizing health economic aspects. *Clinical Nutrition (Edinburgh, Scotland)*, *34*, 1088–1092. doi:10.1016/j.clnu.2015.02.008

Trapl, M., Enderle, P., Nowotny, M., Teuschl, Y., Matz, K., Dachenhausen, A., & Brainin, M. (2007). Dysphagia bedside screening for acute-stroke patients: The Gugging Swallowing Screen. *Stroke*, *38*, 2948–2952. doi:10.1161/STROKEAHA.107.483933

Warnecke, T., Ritter, M.A., Kroger, B., Oelenberg, S., Teismann, I., Heuschmann, P.U., … Dziewas, R. [Rainer] (2009). Fiberoptic endoscopic Dysphagia severity scale predicts outcome after acute stroke. *Cerebrovascular Diseases (Basel, Switzerland)*, *28*, 283–289. doi:10.1159/000228711

Wästfelt, M., Cao, Y., & Ström, J.O. (2018). Predictors of post-stroke fever and infections: A systematic review and meta-analysis. *BMC Neurology*, *18*, 49. doi:10.1186/s12883-018-1046-z

Yoo, S.H., Kim, J.S., Kwon, S.U., Yun, S.C., Koh, J.Y., & Kang, D.W. (2008). Undernutrition as a predictor of poor clinical outcomes in acute ischemic stroke patients. *Archives of Neurology*, *65*, 39–43. doi:10.1001/archneurol.2007.12

5.7. Pharyngeal electrical stimulation for early decannulation in tracheotomized patients with neurogenic dysphagia after stroke (PHAST-TRAC): a prospective, single-blinded, randomized trial[208]

Rainer Dziewas, Rebecca Stellato, Ingeborg van der Tweel, Ernst Walther, Cornelius J Werner, **Tobias Braun**, Giuseppe Citerio, Mitja Jandl, Michael Friedrichs, Katja Nötzel, Milan R Vosko, Satish Mistry, Shaheen Hamdy, Susan McGowan, Tobias Warnecke, Paul Zwittag, Philip M Bath, on behalf of the PHAST-TRAC investigators

Schlaganfallbedingte Dysphagien sind eine häufige Komplikation, die insbesondere bei schwer betroffenen Patienten beobachtet wird. Häufig ist dann auch eine Tracheotomie zum Schutz der Atemwege notwendig. Da eine Pilotstudie bei tracheotomierten Schlaganfallpatienten zeigen konnte, dass eine pharyngeale Elektrostimulation (PES) die Schluckfunktion verbessern konnte, erfolgte diese multizentrische Studie, um diese Ergebnisse zu verifizieren.

Es wurde eine multizentrische, einfach-verblindete und randomisierte Studie mit 9 teilnehmenden Kliniken in Deutschland, Österreich und Italien durchgeführt (7 Akutkrankenhäuser und 2 Rehabilitationseinrichtungen). Patienten, die nach einem frischen Schlaganfall tracheotomiert werden mussten, wurden in die Behandlungsgruppen 1:1 randomisiert. Sie erhielten entweder eine PES oder eine Scheinstimulation. Bei allen Patienten wurde der Stimulationskatheter auch tatsächlich transnasal eingeführt. Bei den scheinstimulierten Patienten wurde die Basisstation der PES mit einer Simulationsbox statt mit dem Stimulationskatheter verbunden. Die Randomisierung erfolgte mittels eines Computersystems in Blöcken à jeweils 4 Patienten für jede Studieneinrichtung. Die Patienten und der Durchführer der PES waren nicht verblindet. Der primäre Endpunkt wurde durch einen eigenständigen Untersucher der jeweiligen Klinik erhoben, der für die erfolgte Therapie verblindet war. Als primärer Outcomeparameter wurde die Möglichkeit der Dekanülierung 24-72 Stunden nach Behandlung definiert. Die Einschätzung erfolgte mittels FEES-Untersuchung und Anwendung eines standardisierten Dekanülierungsprotokolls, das erforderte, dass kein massiver Speichelaufstau vorlag, dass zumindest ein Spontanschluck in einer Minute vorlag und dass die laryngeale Sensibilität zumindest in geringem Ausmaß vorhanden war. Der letzte Punkt wurde sichergestellt durch die Auslösbarkeit eines laryngealen Adduktionsreflexes bei Berührung laryngealer Strukturen mit dem Endoskop. Es erfolgte eine sequenzielle statistische Analyse für die Überlegenheit der PES für den primären Endpunkt. Eine Zwischenanalyse wurde jeweils geplant nach dem Einschluss von 50 Patienten (bezüglich Futility), nach 70 Patienten und dann nach jeweils 10 Patienten, bis schließlich 140 Patienten erreicht waren. Die Analyse war als Intention-to-treat analyse geplant.

Insgesamt wurden über einen Zeitraum von ca. 2 Jahren insgesamt 81 Patienten eingeschlossen. 69 Patienten erfüllten die Kriterien und wurden entsprechend randomisiert (PES: 35 Patienten; Scheinstimulation: 34 Patienten). Der Median von Beginn der Symptomatik bis zur Randomisierung lag bei 28 Tagen (IQR 19–41; PES 28 [20–49]; Scheinstimulation 28 [18–40]). Danach wurde die die Studie frühzeitig abgebrochen, da sich für den primären Endpunkt eine Überlegenheit der PES gezeigt hatte: In der PES-Gruppe (17 von 35 Patienten [49%]) konnten mehr Patienten dekanüliert werden, als in der Scheinstimulationsgruppe (3 von 34 Patienten [9%]). Die Odds-Ration lag bei 7 (95% Konfidenzintervall 2,41-19,88; p=0.0008). Unerwünschte Ereignisse traten bei jeweils 24 Patienten aus beiden Gruppen auf (69% PES; 71% Scheinstimulation). Es lag kein Unterschied bezüglich des zumindest einmaligen Auftretens schwerwiegender Ereignisse zwischen beiden Gruppen vor (10 Patienten in der PES-Gruppe [29%], 8 Patienten mit Scheinstimulation [23%]; p=0,7851). Sieben Patienten (20%) aus der PES-Gruppe und 3 Patienten (9%) aus der Scheinstimulationsgruppe verstarben während des Studienzeitraums (p= 0,3059). Es wurde kein Zusammenhang zwischen der PES und dem Versterben oder unerwünschten Ereignissen gesehen.

Damit zeigte sich, dass bei Patienten mit Schlaganfall und dadurch bedingter Tracheotomie die PES den Teil der Patienten erhöhte, die dekanüliert werden konnten, insbesondere wenn die PES innerhalb des ersten Monats nach dem initialen Schlaganfall erfolgte. Weitere Studien sollten durchgeführt werden, ob sich der Effekt der frühen Stimulation in anderen Studien bei Schlaganfallpatienten ebenfalls zeigen lässt und ob auch andere Patientenkohorten von einer PES profitieren.

Pharyngeal electrical stimulation for early decannulation in tracheotomised patients with neurogenic dysphagia after stroke (PHAST-TRAC): a prospective, single-blinded, randomised trial

*Rainer Dziewas, Rebecca Stellato, Ingeborg van der Tweel, Ernst Walther, Cornelius J Werner, Tobias Braun, Giuseppe Citerio, Mitja Jandl, Michael Friedrichs, Katja Nötzel, Milan R Vosko, Satish Mistry, Shaheen Hamdy, Susan McGowan, Tobias Warnecke, Paul Zwittag, Philip M Bath, on behalf of the PHAST-TRAC investigators**

Summary

Background Dysphagia after stroke is common, especially in severely affected patients who have had a tracheotomy. In a pilot trial, pharyngeal electrical stimulation (PES) improved swallowing function in this group of patients. We aimed to replicate and extend this single-centre experience.

Methods We did a prospective, single-blind, randomised controlled trial across nine sites (seven acute care hospitals, two rehabilitation facilities) in Germany, Austria, and Italy. Patients with recent stroke who required tracheotomy were randomly assigned to receive 3 days of either PES or sham treatment (1:1). All patients had the stimulation catheter inserted; sham treatment was applied by connecting the PES base station to a simulator box instead of the catheter. Randomisation was done via a computerised interactive system (stratified by site) in blocks of four patients per site. Patients and investigators applying PES were not masked. The primary endpoint was assessed by a separate investigator at each site who was masked to treatment assignment. The primary outcome was readiness for decannulation 24–72 h after treatment, assessed using fibreoptic endoscopic evaluation of swallowing and based on a standardised protocol, including absence of massive pooling of saliva, presence of one or more spontaneous swallows, and presence of at least minimum laryngeal sensation. We planned a sequential statistical analysis of superiority for the primary endpoint. Interim analyses were to be done after primary outcome data were available for 50 patients (futility), 70 patients, and every additional ten patients thereafter, up to 140 patients. Analysis was by intention to treat. This trial is registered with the ISRCTN registry, number ISRCTN18137204.

Findings From May 29, 2015, to July 5, 2017, of 81 patients assessed, 69 patients from nine sites were randomly assigned to receive PES (n=35) or sham (n=34) treatment. Median onset to randomisation time was 28 days (IQR 19–41; PES 28 [20–49]; sham 28 [18–40]). The Independent Data and Safety Monitoring Board recommended that the trial was stopped early for efficacy after 70 patients had been recruited and primary endpoint data for 69 patients were available. This decision was approved by the steering committee. More patients were ready for decannulation in the PES group (17 [49%] of 35 patients) than in the sham group (three [9%] of 34 patients; odds ratio [OR] 7·00 [95% CI 2·41–19·88]; p=0·0008). Adverse events were reported in 24 (69%) patients in the PES group and 24 (71%) patients in the sham group. The number of patients with at least one serious adverse event did not differ between the groups (ten [29%] patients in the PES group *vs* eight [23%] patients in the sham group; OR 1·30 [0·44–3·83]; p=0·7851). Seven (20%) patients in the PES group and three (9%) patients in the sham group died during the study period (OR 2·58 [0·61–10·97]; p=0·3059). None of the deaths or serious adverse events were judged to be related to PES.

Interpretation In patients with stroke and subsequent tracheotomy, PES increased the proportion of patients who were ready for decannulation in this study population, many of whom received PES within a month of their stroke. Future trials should confirm whether PES is beneficial in tracheotomised patients who receive stimulation similarly early after stroke and explore its effects in other cohorts.

Funding Phagenesis Ltd.

Introduction

Post-stroke dysphagia is a common complication of acute stroke affecting up to 80% of patients, with 11–50% of patients still dysphagic after 6 months.[1–3] Post-stroke dysphagia interferes with oral feeding and is associated with dehydration, malnutrition, aspiration pneumonia, prolonged hospital stay, poor long-term outcome, and increased mortality.[4–6] Around 1–2% of all patients with

Lancet Neurol 2018

Published Online August 28, 2018 http://dx.doi.org/10.1016/S1474-4422(18)30255-2

See Online/Comment http://dx.doi.org/10.1016/S1474-4422(18)30312-0

*Investigators listed in the appendix

Department of Neurology, University Hospital Münster, Münster, Germany (Prof R Dziewas MD, Prof T Warnecke MD); **Julius Center for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht, Netherlands** (R Stellato MSc, Prof I van der Tweel PhD); **Zentrum für Neurologie und Neurorehabilitation, Schön Klinik Hamburg Eilbek, Hamburg, Germany** (E Walther MD); **Section Interdisciplinary Geriatrics, Department of Neurology, University Hospital RWTH Aachen University, Aachen, Germany** (C J Werner MD); **Neurologische Klinik, University Hospital Giessen and Marburg GmbH, Giessen, Germany** (T Braun MD); **School of Medicine and Surgery, University of Milan-Bicocca, Milan, Italy** (Prof G Citerio MD); **Neurointensive Care, San Gerardo Hospital, ASST-Monza, Italy** (Prof G Citerio); **Isar-Amper-Klinikum, Klinikum München Ost, Haar, Germany** (M Jandl MD); **Median Klinik Berlin Kladow, Berlin, Germany** (M Friedrichs MD); **Neurologie, Vivantes Klinikum Neukölln, Berlin, Germany** (K Nötzel MD); **Klinik für Neurologie 2, Kepler Universitäts Klinikum, Linz, Austria** (M R Vosko MD); **Department for Clinical**

Research, Phagenesis Limited, Manchester, UK (S Mistry PhD); **Centre for Gastrointestinal Sciences, Faculty of Biology, Medicine and Health, University of Manchester and the Manchester Academic Health Sciences Centre, Manchester, UK** (Prof S Hamdy PhD); **National Hospital for Neurology and Neurosurgery, Therapy and Rehabilitation Services London, London, UK** (S McGowan MSc); **Klinik für Hals-Nasen- und Ohrenheilkunde, Kepler Universitäts Klinikum, Linz, Austria** (Prof P Zwittag, MD); **and Stroke Trials Unit, Division of Clinical Neuroscience, University of Nottingham, Nottingham, UK** (Prof P M Bath)

Correspondence to:
Prof Rainer Dziewas, Department of Neurology, University Hospital Münster, 48149 Münster, Germany
dziewas@uni-muenster.de

See Online for appendix

Research in context

Evidence before this study

We searched PubMed for manuscripts published in English from database inception until May 16, 2018, with the terms "stroke" and "dysphagia" in combination with "treatment", "stimulation", "therapy", "rehabilitation", "tracheotomy", "tracheostomy", or "decannulation". Reference lists from identified reviews and trial reports were also checked for additional trials. We identified four randomised controlled trials and one meta-analysis in which pharyngeal electrical stimulation (PES) was delivered in non-ventilated patients with stroke, with heterogeneous results. However, one single-centre pilot randomised controlled trial, which specifically recruited patients who had had a tracheotomy after a stroke, had been conducted by the University of Münster (Münster, Germany), the lead study site of this Pharyngeal electrical Stimulation for early decannulation in Tracheotomised stroke patients with neurogenic dysphagia (PHAST-TRAC) trial. The patients in the pilot study could not be decannulated after successful weaning from the respirator because of severe and persistent post-stroke dysphagia. PES was significantly associated with improvement of airway protection and remission of dysphagia. Overall, post-stroke dysphagia remains one of the most debilitating complications for patients with stroke in both hospital and community health-care settings, conferring a substantial comorbidity with a six-times increased risk of aspiration pneumonia and three-times increased mortality. These increased risks are brought even more into focus because the Cochrane Database for Therapies in Dysphagia after Stroke has reported little evidence for any available treatments being effective in this disorder.

Added value of this study

In this sham-controlled trial of post-stroke tracheotomised patients with severe dysphagia, PES allowed investigators to designate 17 (49%) of 35 patients as ready for decannulation compared with 3 (9%) of 34 patients in the control group (primary endpoint). A prespecified subgroup analysis revealed that response to PES treatment related to a shorter time from stroke onset to randomisation and a shorter period on mechanical ventilation. After the randomised and open-label parts of the study, 37 (57%) of 65 patients who received PES were ready for decannulation. In four (27%) of 15 patients who did not respond to a single treatment cycle of PES (10 min per day for 3 consecutive days), a second treatment cycle proved to be effective. A post-hoc meta-analysis of results from PHAST-TRAC and the pilot trial, which was done in the same population and with the same outcome measure, showed that treatment effects were similar in the two studies and that patients were more than ten-times more likely to be decannulated with PES than with sham.

Implications of all the available evidence

This study provides evidence that PES is effective in promoting earlier decannulation in patients with stroke who have had a tracheotomy and who have post-stroke dysphagia. The size of the difference between the PES and sham groups suggests that a meaningful change in the ability of clinicians to treat these patients might be possible. However, because of the small sample size and differences in treatment effects in subgroups, further trials are needed to corroborate these findings.

stroke, and 25% of patients with stroke treated in the intensive care unit, require a tracheotomy[7–10] due to severe dysphagia with prolonged insufficient airway protection or the need for long-term ventilation.[11,12] Despite the clinical benefits provided by tracheotomy during the acute stage of the illness,[12] the continuing presence of the cannula once the patient has been successfully weaned from the respirator has negative consequences for timely rehabilitation, patient comfort, number of days in hospital,[10] hospital readmission,[13] and the financial costs of care.[13,14] Further, the presence of a tracheotomy tube at discharge from the intensive care unit is predictive of poorer outcome, in part because of cannula-related complications.[15,16] In patients with stroke, severe dysphagia with related insufficient airway protection is often the main reason why decannulation cannot be done even 3 months after stroke.[12,17]

Few options are available to accelerate weaning from the tracheal cannula.[1] Pharyngeal electrical stimulation (PES) is a novel technique shown to enhance reorganisation of the swallow-related motor cortex, to facilitate activation of corticobulbar pathways, and to increase salivary levels of substance P (a neurotransmitter involved in the control of swallowing).[18–20] Studies that used PES to treat post-stroke dysphagia in unselected patients showed heterogeneous results.[21–23] However, in a single-centre, randomised controlled pilot study[24] of 30 patients with acute stroke who had had a tracheotomy, dysphagia improved enough to enable decannulation in 15 (75%) of 20 patients in the treatment group, whereas only two (20%) of ten patients in the control group showed spontaneous remission sufficient enough to allow for subsequent removal of the tracheal cannula. The Pharyngeal electrical Stimulation for early decannulation in Tracheotomised stroke patients with neurogenic dysphagia (PHAST-TRAC) trial was designed to replicate, validate, and extend this single-centre experience in a larger phase 3 design.[25]

Methods

Study design and participants

PHAST-TRAC was an international, prospective, single-blind, randomised controlled trial with a sequential design.[26] The study included a second part in which non-responding patients in either randomised group were given open-label treatment.

Patients were enrolled across nine sites (seven acute care hospitals, two rehabilitation facilities) in Germany, Austria,

and Italy. Details of the study protocol and statistical analysis plan have been published previously.[25]

Patients were eligible for study participation if they were older than 18 years, had presented with a supratentorial stroke (haemorrhagic or ischaemic), were mechanically ventilated for at least 48 h after stroke, were successfully weaned from mechanical ventilation but remained with a tracheotomy, had been free of sedation for at least 3 days at the time of first decannulation screening, scored at least –1 points or more on the Richmond Agitation and Sedation Scale,[27] and could not be decannulated because of severe dysphagia. Patients' readiness for decannulation was assessed twice over 24–72 h and a minimum of 10 days after the stroke using fibreoptic endoscopic evaluation of swallowing (FEES). The presence of massive pooling of saliva, fewer than 1 spontaneous swallows per min, or no sensation elicited by endoscope contact with the laryngeal vestibule (for details of the algorithm see appendix) meant that patients were not ready for decannulation.[28] Key exclusion criteria were infratentorial stroke, pre-existing dysphagia, pre-existing disease that typically causes dysphagia (eg, Parkinson's disease or motor neuron disorders), participation in any other study potentially affecting the outcome of PES, presence of a cardiac pacemaker or an implantable defibrillator, nasal deformity or previous oesophageal surgery, any other circumstance in which placement of a standard nasogastric tube would be deemed unsafe, need for high levels of oxygen supply (>2 L/min), requirement for emergency treatment, or less than 3 months' life expectancy (for a complete list of inclusion and exclusion criteria see appendix).

The study protocol was approved by all relevant national competent authorities (Federal Institute for Drugs and Medical Devices, Germany; Austrian Agency for Health and Food Safety Ltd, Austria; and Ministry of Health, Italy) and local ethics committees for the participating sites, and all patients or their legal representatives provided written informed consent.

A steering committee was responsible for the design, conduct, and reporting of the study. Interim-analyses were reviewed by an independent data and safety monitoring board (IDSMB) after random allocation of 50 and 70 patients, respectively (appendix). Pre-specified stopping rules are given below (statistical analysis).

Randomisation and masking

Patients were randomly assigned to receive PES or sham treatment (1:1) via a computerised interactive wireless randomisation system (IWRS) that applied randomisation stratified by study site in blocks of four patients per site. At each trial site, the randomisation procedure was obtained from the IWRS by a group of investigators responsible only for treatment application. All other investigators and health-care workers not involved in treatment were masked. Conversely, treating investigators were not involved in any outcome assessment or any other study-related activities, such as patient recruitment or dysphagia assessment before or after randomisation. As with many device studies, masking of patients could not be guaranteed because, in principle, patients could feel whether PES was applied. In all other aspects, PES and the sham condition were kept as similar as possible. PES or sham stimulation had to be commenced within 24 h of randomisation.

Procedures

For the study intervention (PES), we used a commercial device (Phagenyx, Phagenesis Ltd, Manchester, UK), which comprises a nasogastric feeding catheter that houses stimulation ring-electrodes and a computerised base station that delivers stimulation in the range 1–50 mA at 5 Hz. In all patients, the stimulation catheter was placed before randomisation. The catheter was inserted via the nose to an aboral depth related to the patient's height so that the pair of treatment ring electrodes located on the outer surface of the catheter were adjacent to the pharynx. A coloured zone on the outer catheter surface and visible at the nares also aided correct placement and easy confirmation of correct electrode depth. Duration of catheter insertion procedure (in min), ease of catheter insertion (rated on a scale from 1 [very difficult] to 7 [very easy]), and electrode depth from nostrils (in cm) were captured.

In all patients, PES or sham stimulation was given on three consecutive days for 10 min each day. The current intensity (mA) at which PES treatment was delivered was individually adjusted and optimised at every session by the health-care worker interacting with the touchscreen on the base station in response to patient responses. This treatment optimisation procedure involved increasing the current intensity incrementally from 1 mA to detect the perceptual threshold (PT; patient first aware of stimulation) and then to the maximum tolerated threshold (MTT; patient no longer wants the current to be increased further) intensity levels three-times each. Thereafter, the optimal treatment intensity was automatically calculated by the base station with the use of average values of the three trials according to the formula $PT + 0{\cdot}75 \times (MTT–PT)$.[21]

In the sham group, the optimisation procedure was imitated as closely as possible to mitigate any bias or effect of time spent interacting with the patient during PES, but no current was applied. To this end, for patients in the sham group, the base station was connected to a small patient simulator box instead of the stimulation catheter placed in the patient. The patient simulator box allowed the treating investigator to interact with the base station as if the stimulation catheter were connected and a real stimulation was about to be delivered. Moreover, in both groups, the connecting ends of the stimulation catheter, base station, and patient simulator were hidden inside a disposable masking pouch to reduce the risk of bias or

unmasking (see appendix for the setup for the PES and sham treatments). After the treatment optimisation procedure, the treating investigator then delivered 10 min of PES or sham stimulation.

After randomised treatment, the primary outcome was assessed at 24–72 h after the last stimulation. All patients who had not responded (ie, showing persistent dysphagia that required the tracheal cannula to be left in situ with the tracheal cuff inflated), irrespective of treatment assignment, were offered open-label treatment with PES. 24–72 h after the last open-label PES treatment, outcome was assessed again with the same FEES-based decannulation algorithm.

Outcomes

The primary endpoint of the study was readiness for decannulation after 3 days of PES treatment, assessed with the FEES-based algorithm.[28] Readiness for decannulation could be clinically followed by either immediate removal of the tracheal tube or cuff deflation. Investigators assessing the primary endpoint were masked to treatment assignment; a separate investigator was used at each site. Investigators from all sites who were responsible for outcome assessment were trained in the use of the FEES-based algorithm. Additionally, to ensure that the decannulation procedure was correctly applied at the study sites, all FEES videos of the primary endpoint assessment were anonymised and adjudicated by an independent FEES Review Board (SMG, TW, PZ) who were not involved in any other study-related activity. Results of the board's ratings were used in post-hoc analyses and not communicated with the sites during the conduct of the trial.

Secondary endpoints were treatment effect in delayed (patients who were judged not ready for decannulation after the initial sham treatment and who were subsequently given open-label PES treatment) and retreated (patients who were judged not ready for decannulation after the initial PES treatment and who were subsequently given a second cycle of open-label PES treatment) patients, necessity of recannulations (at day 2 and during follow-up of 30 days or until discharge, whichever was first), PES treatment parameters, dysphagia scores (dysphagia severity rating scale [DSRS]; functional oral intake scale [FOIS]; appendix), severity of stroke (modified Rankin Scale and National Institutes of Health Stroke Scale scores; at day 2, during follow-up of 30 days or until discharge, whichever was first), length of stay on different levels of care, speech and language therapy management plan, and number and type of adverse events, including adverse events related to treatment or the device (base station or catheter).[22,29]

In a post-hoc analysis, the binary FEES scores (primary endpoint ratings from the local investigators and independent FEES review board) were transformed into an ordered categorical outcome ranging from 0 (none of the three items present) to 3 (all three items present).

Statistical analysis

We performed group sequential monitoring of cumulative data using a triangular test.[26] We set the maximum number of patients, at which the upper and lower decision boundaries met, as 140 with the aim of being able to detect an absolute difference between the groups of 25%, assuming a frequency of decannulation in the sham group of 20%, an overall type 1 error of 0·05, and a power of 0·80 (the 90th percentile of the required sample size was estimated to be 126 patients). We planned to do interim analyses after primary outcome data were available for 50 patients (futility), 70 patients, and every additional ten patients thereafter, up to 140 patients. Decision rules allowed stopping of the trial in the following circumstances:[25] futility—ie, where the difference between treatment groups was very unlikely to be equal to or to exceed 25%, to be done at 50 patients; and superiority of PES treatment—ie, if the readiness for decannulation was more common with PES and equal to or greater than an absolute difference between groups of 25%, to be done at 70, 80, 90, 100, 110, 120, 130, or 140 patients.

We considered patients who did not reach a primary outcome to be not ready for decannulation. For sensitivity purposes, we assessed the heterogeneity of the treatment effect on the primary outcome in prespecified subgroups (age, sex, stroke type, time from stroke onset to randomisation, duration of mechanical ventilation, baseline stroke severity, and stimulation intensity) by adding an interaction term and using exact inference for logistic regression (LogXact11, Cytel Inc, Cambridge, MA, USA).[30]

We analysed other outcomes using Fisher's exact test for binary data, Mann-Whitney *U* test for ordinal data, and Student's *t* test (pooled) for continuous data. We analysed regressions using binary logistic regression, Cox regression, and multiple linear regression. We used Kaplan-Meier analysis on length of stay data. The nominal level of significance for all analyses, including interaction testing, was $p<0·05$. We made no adjustment for multiplicity of testing for secondary analyses, and all analyses were by intention to treat. Statistical analyses were done by the University Medical Centre Utrecht (sequential analysis of the primary endpoint using PEST 4.4)[31] and Cytel Inc (all other analyses using R project software, version R-3.4.1).[32]

For the post-hoc meta-analysis, we did an electronic search for similar trials in PubMed, with the terms "stroke" and "dysphagia" in combination with "treatment", "stimulation", "therapy", "rehabilitation", "tracheotomy", "tracheostomy", or "decannulation" from database inception to May 16, 2018. Only trials that used PES in tracheotomised stroke patients were selected.

The trial was registered with the ISRCTN registry, number ISRCTN18137204.

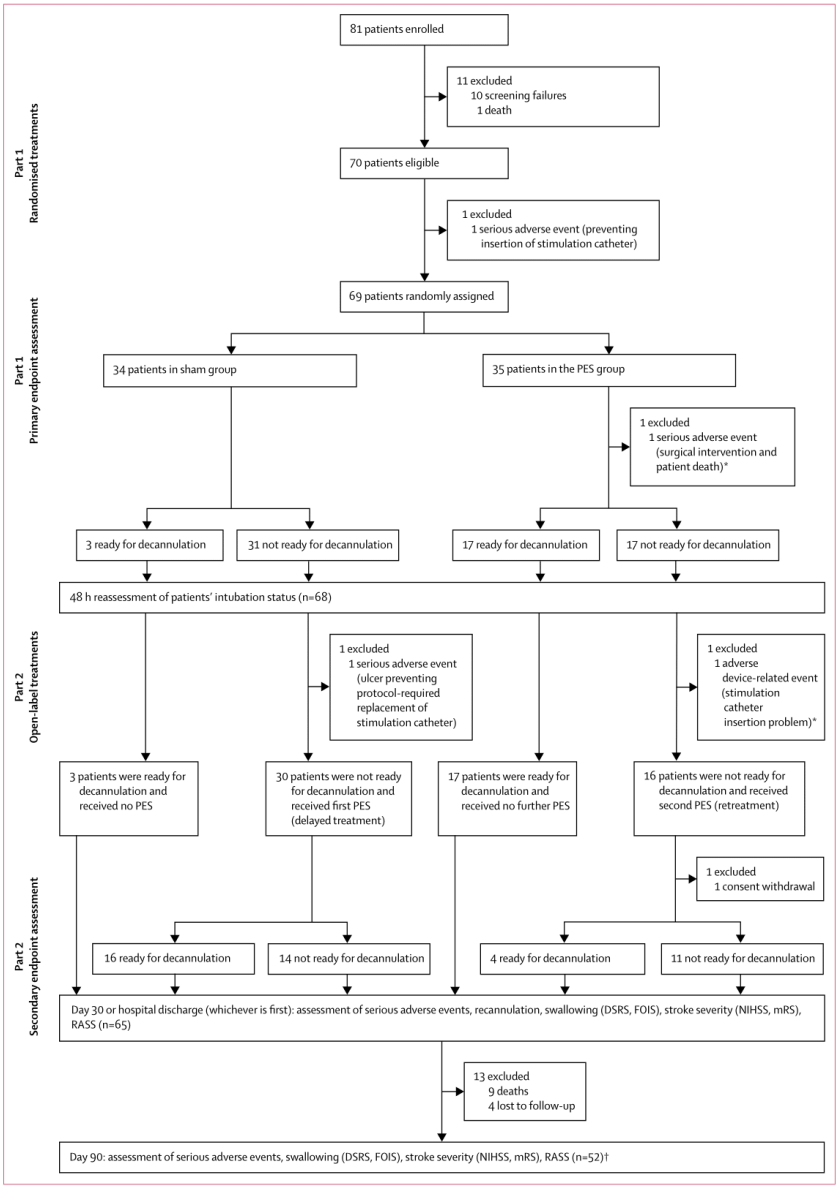

Figure 1: **Trial profile**
DSRS=Dysphagia Severity Rating Scale. FOIS=Functional Oral Intake Scale. mRS=modified Rankin Scale. NIHSS=National Institutes of Health Stroke Scale. PES=pharyngeal electrical stimulation. RASS=Richmond Agitation and Sedation Scale. *Adjudicated as treatment failure (primary endpoint). †Data for RASS were collected during follow-up in this study but are not presented in table 2 as data for NIHSS and mRS are more relevant metrics of patient cognition and recovery at these time points.

	PES group	Sham group
Patients	35	34
Age, years (SD)	61·7 (13·0)	66·8 (10·3)
Sex		
Women	11 (31%)	14 (41%)
Men	24 (69%)	20 (59%)
mRS premorbid >0	1 (3%)	2 (6%)
mRS >4	34 (100%)	33 (97%)
Medical history		
Hypertension	23 (66%)	26 (77%)
Hyperlipidaemia	1 (3%)	3 (9%)
Diabetes	3 (9%)	5 (15%)
Atrial fibrillation	3 (9%)	2 (6%)
Previous stroke or TIA	7 (20%)	3 (9%)
Smoking	5 (14%)	3 (9%)
Onset to randomisation, days (range)	28·0 (21·0–50·5)	28·0 (19·3–41·8)
Ventilation, days (range)	15·0 (9·5–23·0)	13·5 (9·3–22·0)
Feeding status, PEG	5 (23%)	4 (18%)
NIHSS, out of 24 (SD)	17·6 (5·0)	17·5 (4·3)
Ischaemic stroke	27 (77%)	22 (65%)
Haemorrhagic stroke	8 (23%)	12 (35%)
Lesion side, right	17 (49%)	16 (47%)
DSRS, out of 12 (SD)	12 (0)	12 (0)
FOIS, out of 7 (SD)	1 (0)	1 (0)

Data are n (%), mean (SD), or median (IQR) unless otherwise specified. Number of patients enrolled (randomised) per site: Münster, Germany=40 (40); Hamburg, Germany=14 (8); Aachen, Germany=8 (6); Monza, Italy=6 (5); Munich, Germany=4 (4); Berlin Median, Germany=3 (2); Berlin Vivantes, Germany=2 (2); Linz, Austria=2 (2); Giessen, Germany=2 (0). PES=pharyngeal electrical stimulation. mRS=modified Rankin Scale. TIA=transient ischaemic attack. PEG=percutaneous endoscopic gastrostomy. NIHSS=National Institutes of Health Stroke Scale. DSRS=dysphagia severity rating scale. FOIS=functional oral intake scale. There were no significant differences between the study groups.

Table 1: **Baseline characteristics of patients randomly assigned to each group**

Role of the funding source

The study was sponsored by Phagenesis Ltd. The sponsor was involved in the design of the study, and contributed to data interpretation and the writing of the manuscript. It also financially compensated sites for data collection, a clinical research organisation (FAKKEL, Belgium; for further details see appendix) for study management and source data verification, and University Medical Centre Utrecht (Utrecht, Netherlands) and Cytel Inc (Cambridge, MA, USA) for data analysis. Interim analyses were reviewed by the IDSMB without involvement of the sponsor or the steering committee. All authors had full access to all data. The corresponding author had final responsibility for the decision to submit for publication.

Results

The study was done between May 29, 2015, and July 5, 2017. A total of 81 patients were assessed for eligibility for the study, of whom 70 patients were found eligible. The stimulation catheter could not be placed in one patient and therefore 69 of 70 patients who were successfully screened were randomly assigned. 35 patients were assigned to receive PES and 34 to receive sham stimulation during the masked study part (figure 1). Sequential analysis after 50 patients had reached the primary endpoint showed no futility. After 70 patients, sequential analysis indicated superiority in favour of PES treatment (appendix) such that the IDSMB recommended that the trial should stop. The one patient in whom the stimulation catheter could not be placed and who was not randomly assigned to a study group was initially adjudicated as a treatment failure and only later, when this mistake was detected, he was removed from the intention-to-treat population. The steering committee reviewed the same data and IDSMB advice to stop and agreed with the decision (appendix).

25 (36%) of 69 patients were women and the mean age was 64·2 years (SD 11·9). 49 (71%) of 69 patients had an ischaemic stroke, and 20 (29%) of 69 patients had an intracerebral haemorrhage. The mean National Institutes of Health Stroke Scale (NIHSS) score was 17·5 (4·6), and patients were randomly assigned at a median of 28 days (IQR 19–41) after ictus and after 15 days (IQR 9–22) of mechanical ventilation (table 1).

17 (49%) of 35 patients in the PES group and three (9%) of 34 patients in the sham group were judged to be ready for decannulation after the masked first part of the study (odds ratio [OR] 7·00 [95% CI 2·41–19·88]; p=0·00082; table 2). In predefined subgroups, significant treatment-by-subgroup interactions were present favouring treatment in patients treated earlier after stroke or with a shorter duration of mechanical ventilation (figure 2). After the decision to decannulate, 14 (82%) of 17 patients in the PES group were decannulated and the cuff was permanently deflated without decannulation in the other three (table 2).

None of the patients who had decannulation required recannulation over the next 48 h or during their follow-up period up to hospital discharge. The decannulation assessment occurred mainly 24–48 h after the third day of PES or sham (n=24 in the PES group, n=21 in the sham group); the remaining patients were assessed in a time window of 48–72 h (n=10 in the PES group, n=13 in the sham group).

During the unmasked (open-label), second part of the study, 17 (49%) of 35 patients from the PES group who did not reach primary endpoint remained. Of these, one (3%) patient experienced an adverse event, which precluded replacement of the stimulation catheter, and one (3%) patient withdrew. 15 (43%) patients received a second (retreatment) cycle of PES; subsequently, four (27%) of 15 retreated patients were judged to be ready for decannulation. 30 patients from the sham group received a first (delayed) cycle of PES, with a further one patient (3%) withdrawing owing to identification of a bleeding gastric ulcer precluding catheter insertion. 16 (53%) of these 30 patients were judged to be ready for decannulation

after 3 days of PES (table 2). Taking into account both the randomised and open-label parts of the study, and after at least one course of PES, a total of 37 (57%) of 65 patients became ready for decannulation 24–72 h after PES. Clinical dysphagia scores (DSRS, FOIS) and stroke severity (NIHSS and modified Rankin Scale) did not differ between the initial treatment groups (table 2).

During the randomised part of the study, mean optimised PES stimulation intensity was 33·6 mA (SD 8·3) and mean perceptual threshold was 15·2 mA (9·3; appendix). The mean time needed for the initial catheter insertion was 8·7 min (SD 9·3; median 6·4 min, range 2–30), and 44 (70%) of 63 evaluated catheter placements were judged to be very easy (rated as a score of 6 or 7; appendix). The stimulation catheter could not be inserted in two patients (one before randomisation, one before the second treatment cycle).

While at baseline and directly after study intervention, most patients needed to be treated in the intensive or intermediate care unit, during follow-up the level of care decreased in most of the patients without any difference between the PES group and sham group (table 2).

During the follow-up period, only 20 speech and language therapy reports from nine patients and two centres were obtained. These reports documented a gradual improvement over time in five patients, whereas in four patients no oral intake was possible. Since these data did not add information to the documented dysphagia scores, they have not been analysed or presented in detail.

Adverse events were reported in 24 (69%) patients in the PES group and 24 (71%) patients in the sham group. Seven (20%) patients in the PES group, three (9%) patients in the sham group, and one patient who had yet to be randomly assigned died during the study (table 3). Seven of these deaths occurred more than 30 days after randomisation. None of the deaths were judged by the IDSMB to be related to PES treatment or to the investigational device. The number of patients with at least one serious adverse event did not differ between the two groups (ten [29%] of 35 patients in the PES group *vs* eight [23%] of 34 patients in the sham group; OR 1·30 [95% CI 0·44–3·83]; p=0·7851). No serious adverse event occurred during the open-label part in the 15 patients who had also received PES during the randomised part of the trial (retreated patients); no serious adverse events related to PES treatment or to the investigational device were observed in the entire study (table 3). 12 non-serious device-related adverse events were observed in eight different patients (table 3). Most notably, in three patients, technical problems with the stimulation device occurred that were later resolved. Additionally, in one patient the stimulation catheter could not be placed and another patient did not tolerate 10 min of PES. Finally, one patient experienced discomfort during stimulation and removed the stimulation catheter prematurely (appendix).

Post-hoc, treatment responders (patients deemed ready for decannulation) and non-responders (patients deemed

	n	PES group*	Sham group	Odds ratio or median difference (95% CI)	p value
Primary outcome (randomised part of the study)					
Patients	69	35	34		
Ready for decannulation after PES or sham (primary outcome)		17 (49%)	3 (9%)	7·00 (2·41–19·88)	0·0008
Removal of the tracheal tube†		14 (82%)	1 (33%)	9·33 (0·62–139·57)	0·1404
Deflation of the tube-cuff†		3 (18%)	1 (33%)	0·43 (0·03–6·41)	0·5088
Secondary outcomes (open-label part of the study)					
Patients	45	15	30		
Ready for decannulation after open-label treatment‡	20 (44%)	4 (27%)	16 (53%)	0·32 (0·08–1·23)	0·1185
Removal of the tracheal tube†	17 (38%)	3 (20%)	14 (47%)	0·29 (0·07–1·22)	0·1097
Deflation of the tube-cuff†	3 (7%)	1 (7%)	2 (7%)	1·00 (0·08–12·00)	1·0000
Recannulation within 48 h		0	0	..	..
Recannulation within 30 days or hospital discharge (whichever is first)		0	0	..	..
DSRS					
Day 2 (SD)	60	30, 10·6 (2·4)	30, 10·4 (2·7)	0·27 (–1·05 to 1·59)	0·6873
Day 30 or hospital discharge, whichever is first (SD)	50	25, 8·0 (4·6)	25, 8·9 (3·3)	–0·88 (–3·17 to 1·41)	0·4437
Day 90 (SD)	53	27, 4·6 (5·3)	26, 5·7 (5·1)	–1·10 (–3·97 to 1·77)	0·4449
FOIS					
Day 2 (SD)	61	31, 1·7 (1·2)	30, 1·9 (1·4)	–0·191 (–0·878 to 0·495)	0·5789
Day 30 or hospital discharge, whichever is first (SD)	50	25, 3·0 (2·4)	25, 2·5 (1·7)	0·560 (–0·61 to 1·73)	0·3407
Day 90 (SD)	53	27, 4·6 (2·6)	26, 3·9 (2·5)	0·745 (–0·660 to 2·150)	0·2922
NIHSS					
Baseline (SD)	68	34, 17·6 (5·0)	34, 17·5 (4·3)	0·118 (–2·129 to 2·364)	0·9170
Day 2 (SD)	47	24, 15·6 (4·5)	23, 15·7 (6·4)	–0·027 (–3·287 to 3·233)	0·9867
Day 30 or hospital discharge, whichever is first (SD)	48	24, 14·0 (5·0)	24, 13·8 (5·9)	0·292 (–2·865 to 3·448)	0·8533
Day 90 (SD)	16	8, 10·1 (9·2)	8, 16·9 (8·6)	–6·750 (–16·281 to 2·781)	0·1510
mRS					
Baseline (SD)	68	34, 5·0 (0·0)	34, 5·0 (0·2)	0·029 (–0·029, 0·088)	0·3210
Day 2 (SD)	61	31, 4·6 (1·3)	30, 4·6 (1·3)	0·078 (–0·570, 0·727)	0·8094
Day 30 or hospital discharge, whichever is first (SD)	54	28, 4·8 (0·5)	26, 4·7 (0·5)	0·091 (–0·163 to 0·345)	0·4769
Day 90 (SD)	51	26, 4·1 (0·8)	25, 4·3 (1·0)	–0·203 (–0·730 to 0·324)	0·4421
Level of care					
Baseline					
Patients	65	32	33		
Intensive care unit		8 (25%)	7 (21%)	1·24 (0·39–3·93)	0·7746
Intermediate care unit		21 (66%)	23 (70%)	0·83 (0·29–2·35)	0·7944
Normal ward		3 (10%)	3 (10%)	1·03 (0·19–5·55)	1·0000
Level of care					
Day 2					
Patients	50	25	25		
Intensive care unit		3 (12%)	1 (4%)	3·27 (0·32–33·84)	0·6092
Intermediate care unit		15 (60%)	16 (64%)	0·84 (0·27–2·65)	1·0000
Normal ward		7 (28%)	8 (32%)	0·83 (0·25–2·78)	1·0000

(Table 2 continues on next page)

	PES group	Sham group
Patients	35	34
Age, years (SD)	61·7 (13·0)	66·8 (10·3)
Sex		
Women	11 (31%)	14 (41%)
Men	24 (69%)	20 (59%)
mRS premorbid >0	1 (3%)	2 (6%)
mRS >4	34 (100%)	33 (97%)
Medical history		
Hypertension	23 (66%)	26 (77%)
Hyperlipidaemia	1 (3%)	3 (9%)
Diabetes	3 (9%)	5 (15%)
Atrial fibrillation	3 (9%)	2 (6%)
Previous stroke or TIA	7 (20%)	3 (9%)
Smoking	5 (14%)	3 (9%)
Onset to randomisation, days (range)	28·0 (21·0–50·5)	28·0 (19·3–41·8)
Ventilation, days (range)	15·0 (9·5–23·0)	13·5 (9·3–22·0)
Feeding status, PEG	5 (23%)	4 (18%)
NIHSS, out of 24 (SD)	17·6 (5·0)	17·5 (4·3)
Ischaemic stroke	27 (77%)	22 (65%)
Haemorrhagic stroke	8 (23%)	12 (35%)
Lesion side, right	17 (49%)	16 (47%)
DSRS, out of 12 (SD)	12 (0)	12 (0)
FOIS, out of 7 (SD)	1 (0)	1 (0)

Data are n (%), mean (SD), or median (IQR) unless otherwise specified. Number of patients enrolled (randomised) per site: Münster, Germany=40 (40); Hamburg, Germany=14 (8); Aachen, Germany=8 (6); Monza, Italy=6 (5); Munich, Germany=4 (4); Berlin Median, Germany=3 (2); Berlin Vivantes, Germany=2 (2); Linz, Austria=2 (2); Giessen, Germany=2 (0). PES=pharyngeal electrical stimulation. mRS=modified Rankin Scale. TIA=transient ischaemic attack. PEG=percutaneous endoscopic gastrostomy. NIHSS=National Institutes of Health Stroke Scale. DSRS=dysphagia severity rating scale. FOIS=functional oral intake scale. There were no significant differences between the study groups.

Table 1: **Baseline characteristics of patients randomly assigned to each group**

Role of the funding source

The study was sponsored by Phagenesis Ltd. The sponsor was involved in the design of the study, and contributed to data interpretation and the writing of the manuscript. It also financially compensated sites for data collection, a clinical research organisation (FAKKEL, Belgium; for further details see appendix) for study management and source data verification, and University Medical Centre Utrecht (Utrecht, Netherlands) and Cytel Inc (Cambridge, MA, USA) for data analysis. Interim analyses were reviewed by the IDSMB without involvement of the sponsor or the steering committee. All authors had full access to all data. The corresponding author had final responsibility for the decision to submit for publication.

Results

The study was done between May 29, 2015, and July 5, 2017. A total of 81 patients were assessed for eligibility for the study, of whom 70 patients were found eligible. The stimulation catheter could not be placed in one patient and therefore 69 of 70 patients who were successfully screened were randomly assigned. 35 patients were assigned to receive PES and 34 to receive sham stimulation during the masked study part (figure 1). Sequential analysis after 50 patients had reached the primary endpoint showed no futility. After 70 patients, sequential analysis indicated superiority in favour of PES treatment (appendix) such that the IDSMB recommended that the trial should stop. The one patient in whom the stimulation catheter could not be placed and who was not randomly assigned to a study group was initially adjudicated as a treatment failure and only later, when this mistake was detected, he was removed from the intention-to-treat population. The steering committee reviewed the same data and IDSMB advice to stop and agreed with the decision (appendix).

25 (36%) of 69 patients were women and the mean age was 64·2 years (SD 11·9). 49 (71%) of 69 patients had an ischaemic stroke, and 20 (29%) of 69 patients had an intracerebral haemorrhage. The mean National Institutes of Health Stroke Scale (NIHSS) score was 17·5 (4·6), and patients were randomly assigned at a median of 28 days (IQR 19–41) after ictus and after 15 days (IQR 9–22) of mechanical ventilation (table 1).

17 (49%) of 35 patients in the PES group and three (9%) of 34 patients in the sham group were judged to be ready for decannulation after the masked first part of the study (odds ratio [OR] 7·00 [95% CI 2·41–19·88]; p=0·00082; table 2). In predefined subgroups, significant treatment-by-subgroup interactions were present favouring treatment in patients treated earlier after stroke or with a shorter duration of mechanical ventilation (figure 2). After the decision to decannulate, 14 (82%) of 17 patients in the PES group were decannulated and the cuff was permanently deflated without decannulation in the other three (table 2).

None of the patients who had decannulation required recannulation over the next 48 h or during their follow-up period up to hospital discharge. The decannulation assessment occurred mainly 24–48 h after the third day of PES or sham (n=24 in the PES group, n=21 in the sham group); the remaining patients were assessed in a time window of 48–72 h (n=10 in the PES group, n=13 in the sham group).

During the unmasked (open-label), second part of the study, 17 (49%) of 35 patients from the PES group who did not reach primary endpoint remained. Of these, one (3%) patient experienced an adverse event, which precluded replacement of the stimulation catheter, and one (3%) patient withdrew. 15 (43%) patients received a second (retreatment) cycle of PES; subsequently, four (27%) of 15 retreated patients were judged to be ready for decannulation. 30 patients from the sham group received a first (delayed) cycle of PES, with a further one patient (3%) withdrawing owing to identification of a bleeding gastric ulcer precluding catheter insertion. 16 (53%) of these 30 patients were judged to be ready for decannulation

	n	PES group*	Sham group	Odds ratio or median difference (95% CI)	p value
(Continued from previous page)					
Day 10					
Patients	24	13	11		
Intensive care unit		2 (15%)	1 (9%)	1·82 (0·14–23·25)	1·0000
Intermediate care unit		4 (31%)	5 (46%)	0·53 (0·10–2·84)	0·6752
Normal ward		7 (54%)	5 (46%)	1·40 (0·28–7·02)	1·0000
Day 30					
Patients	14	7	7		
Intensive care unit		0	0	-	-
Intermediate care unit		2 (29%)	1 (14%)	2·40 (0·16–34·93)	1·0000
Normal ward		5 (71%)	6 (86%)	0·42 (0·03–6·06)	1·0000

Data are n (%), unless otherwise specified. Data for DSRS, FOIS, NIHSS, and mRS are n, mean (SD). PES=pharyngeal electrical stimulation. DSRS=dysphagia severity rating scale. FOIS=functional oral intake scale. NIHSS=National Institutes of Health Stroke Scale. mRS=modified Rankin Scale. FEES=fibreoptic endoscopic evaluation of swallowing. *One patient in the PES group had an adverse event that was not related to treatment, which occurred on the third day of PES and required transfer to another hospital for surgery; as a result, FEES assessment was not possible and they were assigned to no decannulation. †Prespecified statistical comparison within the subgroup of patients reaching the primary endpoint. ‡These data relate only to the open-label part of the study where all non-responders were given PES. Responders were not given a second cycle of PES; they entered follow-up.

Table 2: **Primary and secondary outcomes in the randomised and open-label parts of the trial**

not ready for decannulation) were compared for different outcomes. We found a significant difference in dysphagia scores between these groups, favouring treatment responders both at discharge and 90-day follow-up (appendix). Similarly, treatment responders were discharged significantly earlier than treatment non-responders (median length of stay after PES was 14 days [95% CI 12–15] in responders *vs* 36 days [16–102] in non-responders; p=0·0006; appendix). Finally, when analysing stimulation intensities, patients who responded to PES had lower PES perceptual threshold levels than those who did not respond (12·1 mA *vs* 18·5 mA); PES tolerance levels (37·0 mA *vs* 42·3 mA) and PES stimulation intensities (31·2 mA *vs* 36·0 mA) did not differ significantly between the groups (appendix).

To address the issue of masking, we did a post-hoc analysis that reanalysed study outcome based on the findings of the independent FEES review board. In this analysis, PES was also associated with an increased proportion of patients who were ready for decannulation (ten [29%] of 35 patients in the PES group *vs* two [6%] of 34 patients in the sham group; OR 6·40 [95% CI 1·28–31·88]; p=0·0234; appendix), although the treatment effect was smaller than in the primary outcome analysis.

Another post-hoc analysis was done to assess in more detail the differences in swallowing function between the PES and sham groups and to account for differences in patient recruitment (both in numbers and clinical characteristics) across study sites. PES was associated with a shift to fewer FEES markers of dysphagia (median difference –1·0 [95% CI –2·0 to 0·0]; p=0·0180), whereas the proportion of patients with worst scores were similar between groups (ten [29%] of 35 patients in the PES and nine [31%] of 34 patients in the sham group; appendix). Patients from the study site with highest recruitment (Münster, Germany) differed from patients in other sites in that they had less severe stroke (mean NIHSS 16·3 [SD 3·9] *vs* 19·3 [5·1]; p=0·0017), shorter times for onset to randomisation (median 23·50 days [IQR 18·75–28·25] *vs* 53·00 [30·00–66·00]; p<0·0001), and shorter times on a ventilator (mean 10·9 days [SD 4·7] *vs* 33·2 [23·4]; p<0·0001). A multiple variable model predicting response and comprising PES, age, onset-to-randomisation time, duration of ventilation, NIHSS, and recruitment site (Münster) found that only PES (p=0·0066) and onset-to-randomisation time (p=0·0361) were significantly related to outcome (appendix).

Post-hoc summary meta-analysis of results from the randomised part of PHAST-TRAC and the only other related trial, done at the study site that also had the largest number of participants in PHAST-TRAC, in the same population and using the same outcome measure,[24] showed that treatment effects were similar between these two trials and that PES was associated with a more than 10-times chance of decannulation as compared to sham (appendix).

Discussion

In this trial, PES allowed investigators to rate 17 (49%) of 35 patients as ready for decannulation compared with 3 (9%) of 34 patients in the sham group. Response to treatment appeared to be related to a shorter time from stroke onset to randomisation and shorter period on mechanical ventilation. After both the randomised and open-label parts of the study, 37 (57%) of 65 patients who received PES were ready for decannulation and in a post-hoc analysis patients responding to PES were discharged from hospital significantly earlier than non-responders.

The effect size of PES in this study was in keeping with that of the earlier single-centre pilot trial,[24] as summarised in the meta-analysis. Similarly, the low rate of spontaneous recovery in the sham group is compatible with the Decannulation and Functional Outcome After Tracheostomy in Patients with Severe Stroke (DECAST) cohort study,[17] in which only 14 (26%) of 53 patients with stroke who had a tracheotomy could be decannulated within 3 months of stroke.

The association between treatment efficacy and short time to treatment is presumably related to the development of critical illness dysphagia due to critical illness polyneuropathy (CIP) and myopathy (CIM) in patients with prolonged intensive-care unit treatment and mechanical ventilation.[33,34] Apart from stroke-related impairment of the central swallowing network, CIP and CIM will damage swallowing-related cranial nerves and muscles, respectively. Further, PES is dependent on intact laryngeal and pharyngeal sensory afferent pathways, so that severe polyneuropathy might interfere with its known effect on brain plasticity. Although the present study did not include neurophysiological assessments, the higher

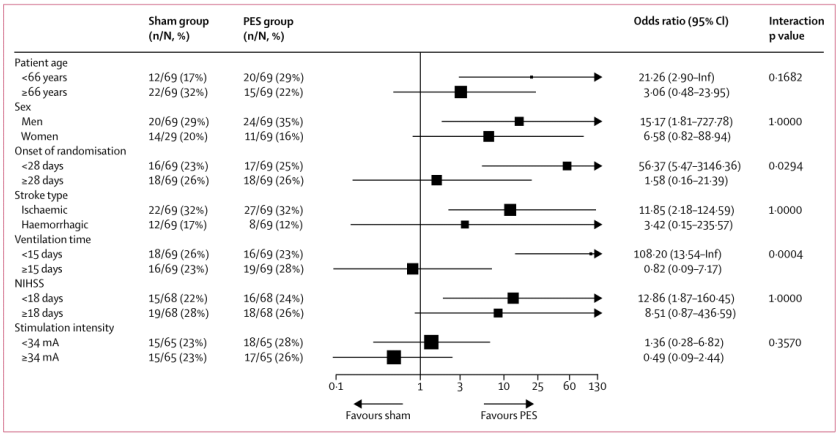

Figure 2: **Forest plot of treatment by subgroup interactions**
Data for variables are presented dichotomised using the median of each variable except for stimulation intensity where the mean was used. Only onset-to-randomisation time and ventilation time were significantly related to treatment success. PES=pharyngeal electrical stimulation. Inf=infinity. NIHSS=National Institutes of Health Stroke Scale. mA=milliampere.

sensory thresholds observed in patients who were not deemed ready for decannulation compared with successfully treated ones supports the notion of impaired sensory feedback as an important reason for treatment failure.

Another key finding of this trial was that a second cycle of PES proved to be effective in four (27%) of 15 patients still requiring a tracheal cannula after having already received a treatment cycle of PES. This result is in line with an open-label cohort study[35] and suggests that patients who do not respond to one cycle (3 days) of treatment should be treated again.

Apart from confirming the present results, future trials should therefore particularly focus on the substantially more difficult-to-treat group of patients with stroke who have had a tracheotomy and who have longer times from stroke onset (>28 days) and longer times of preceding mechanical ventilation (>15 days). These trials should allow for the possibility of repetitive PES and, ideally, determine potential biomarkers of treatment success.

The positive results of the present study are clearly different from those of the Swallowing Treatment Using Pharyngeal Electrical Stimulation (STEPS) trial,[21] in which PES, applied according to the same treatment paradigm, did not improve swallowing safety (primary endpoint) or clinical dysphagia scores (secondary endpoints) compared with sham in a cohort of 162 patients with stroke who never required ventilation. Although no firm conclusions can be drawn with regard to the reasons for these discrepant findings, some key differences between the trials might help in providing some tentative explanations. First, the STEPS trial recruited patients with stroke who were less severely affected (mean NIHSS <10 *vs* 17·5 in PHAST-TRAC) and many were on a partial oral diet at study inclusion. Second, in STEPS the median onset-to-randomisation time was 11 days compared with 28 days in PHAST-TRAC, which suggests that spontaneous recovery of post-stroke dysphagia might have been more prevalent in STEPS than in PHAST-TRAC. Last, stimulation intensities were different, with a mean of 14·5 mA in STEPS compared with 33·6 mA in PHAST-TRAC, which suggests that PES might have been given at a more effective dose in PHAST-TRAC.

The strength of this phase 3 trial is its multicentre sham-controlled design in a well defined group of patients with stroke, and consistent results across the masked and open-label parts of the study. However, some limitations are apparent. First, although the trial was the largest study of PES in such patients, it was still small, reflecting the adaptive design, which led to a reduction in sample size. Nevertheless, the primary outcome findings are consistent with other results from this study and those from the pilot trial. Second, PES was delivered in a single-blind fashion with the person delivering PES being unmasked. Since both treatment and endpoint assessments were done locally at the sites, bias might have been introduced, although endpoint assessors were masked to patients' randomisation group. To address this concern prospectively, an independent FEES review board masked to recruitment site and treatment

	PES group	Sham group	Non-randomised*
Serious adverse events			
Study total	12 (10 [29%])	9 (8 [24%])	3 (2 [17%])
Before randomisation†	1 (1 [3%])	1 (1 [3%])	3 (2 [17%])
0–1 month after randomisation	3 (3 [9%])	4 (4 [12%])	..
1–3 months after randomisation	8 (7 [20%])	4 (1 [3%])	..
Most commonly observed serious adverse events (≥3 events)			
Pneumonia	2 (2 [6%])	1 (1 [3%])	..
Cardiac arrest	2 (2 [6%])	1 (1 [3%])	..
Sepsis	3 (3 [9%])	4 (4 [12%])	..
Hydrocephalus	2 (2 [6%])	0	1 (1 [8%])
Death	7 (7 [20%])	3 (3 [9%])	1 (1 [8%])
Non-serious adverse events	55 (21 [60%])	50 (21 [62%])	0
Most commonly observed non-serious adverse events (≥3 events)			
Diarrhoea	2 (2 [6%])	4 (4 [12%])	..
Vomiting	6 (4 [11%])	6 (2 [6%])	..
Pneumonia	3 (3 [9%])	6 (5 [15%])	..
Urinary tract infection	8 (7 [20%])	3 (3 [9%])	..
Infection (other)	6 (6 [17%])	4 (3 [9%])	..
Musculoskeletal pain	3 (2 [6%])	0	..
Hypoxia	2 (2 [6%])	1 (1 [3%])	..
Thrombophlebitis	2 (2 [6%])	1 (1 [3%])	..
Adverse device-related events	8 (5 [14%])	4 (3 [9%])	0
Most commonly observed adverse device-related events (≥3 events)			
Medical device complication	6 (5 [14%])	3 (2 [6%])	0
Serious adverse device-related events	0	0	0

Data are number of events (number of patients [%]). PES=pharyngeal electrical stimulation. None of the patient deaths or serious adverse events was judged by the Independent Data and Safety Monitoring Board to be caused by the intervention (PES treatment) or investigational device (Phagenyx base station and catheter). Adverse device-related events, also referred to as device deficiencies, are defined as an inadequacy of the medical device with respect to its identity, quality, durability, reliability, safety, or performance, and includes malfunctions, use errors, and inadequate labelling. See appendix for further details of all adverse events observed during this study. *Non-randomised is defined as patients who were not ultimately randomly assigned to treatment. †Before randomisation is defined as period from date of informed consent to date of randomisation.

Table 3: **Adverse events and serious adverse events**

assignment re-evaluated videos from the primary outcome FEES examination, and their results are compatible with the findings of local investigators. Third, the primary outcome was assessed a few days after the end of treatment, after which open-label treatment was offered to all patients who did not reach the primary outcome, irrespective of their original treatment group. As a result, the effect of randomised treatment on long-term outcomes could not be assessed. Nevertheless, 20 patients benefitted from this additional opportunity for active treatment. Fourth, the mean age of the sham group was numerically higher than that of the treatment group, which might have had a negative effect on the recovery rate in the sham group. However, since the subgroup analysis did not show an interaction between age and PES, this baseline difference is unlikely to have affected the result. Finally, the majority of patients were recruited from one site, and the experience and treatment delivery approach at this site might underpin the primary outcome result. Therefore, a replication of findings, preferentially in a larger trial, would seem desirable. However, early recruitment after stroke rather than the recruitment site appeared to predict response in multiple variable modelling.

In conclusion, in patients with severe post-stroke dysphagia who have had a tracheotomy, PES was safe and superior to sham in improving airway protection and swallowing function and led to higher rates of decannulation. Future trials should confirm these results and explore the effect of PES in other cohorts of patients with a tracheotomy.

Contributors

RD, IvdT, SM, SH, and PMB contributed to the conception and design of the study. RD, EW, CJW, TB, GC, MJ, MF, KN, and MRV enrolled patients in the study and contributed to data collection and verification. SMG, TW, and PZ performed the independent fibreoptic endoscopic evaluation of swallowing video reviews. RS and IvdT analysed the primary endpoint data. RD, SH, and PMB interpreted the data. RD, SH, and SM reviewed adverse events and handled the data for the independent data a nd safety monitoring board (IDSMB) review. RS and IvdT were non-voting members of the IDSMB and were advisers to the scientific committee. RD performed the literature search for the meta-analysis. A scientific design committee (RD, IvdT, SM, SH, and PB) was responsible for the design of the study. A steering committee (PMB [chair] and RD) was responsible for the conduct and reporting of the study and interpretation of the results. RD, SM, SH, and PMB prepared figures and tables and drafted the manuscript, which was critically revised for important intellectual content by RS, IvdT, EW, CJW, TB, GC, MJ, MF, KN, MRV, SMG, TW, and PZ. All authors approved the final version of the manuscript.

Declaration of interests

RD reports reimbursement of travel expenses during the study conduct and his role as principal investigator from Phagenesis Ltd. RD also reports travel reimbursement for scientific committee meetings, reports that the University Hospital Münster received payment per patient for study conduct, reports that the University Hospital Münster received payment per patient within the Pharyngeal electrical stimulation for treatment of neurogenic dysphagia: a European Registry (PHADER), received honoraria for serving as a consultant for Nestlé HealthScience, and worked as non-paid adviser for the company Phagenesis Ltd. RS and IvdT report that Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht received payment for the execution of the sequential analysis and for the participation in the IDSMB reviews as part of the PHAST-TRAC study from Phagenesis Ltd. EW reports reimbursement of travel expenses for training during the study conduct, and reports that Schön Klinik Hamburg Eilbek received payment per patient for study conduct and received payments per patient for the PHADER registry. CJW reports reimbursement of travel expenses for training during the study conduct, and reports that RWTH Aachen University received payment per patient for study conduct. TB reports reimbursement of travel expenses for training during the study conduct, and reports that University Hospital Giessen received payment per patient for study conduct. GC reports that University Hospital Bicocca received payment per patient for study conduct. MJ reports reimbursement of travel expenses for training during the study conduct, and reports that Isar-Amper Klinikum received payment per patient for study conduct. KN reports reimbursement of travel expenses for training during the study conduct, and reports that Hospital Vivantes Neukölln received payment per patient for study conduct. MRV reports reimbursement of travel expenses before and during the study conduct, and reports that Kepler University Hospital received payment per patient for study conduct and received payments per patient for the PHADER registry. MF reports reimbursement of travel expenses tduring the study conduct, and reports that Median Klinik Berlin-Kladow received payment per patient for study conduct. SM reports receiving fees from Phagenesis Ltd as an employee of that company. SH reports owning stocks and shares from Phagenesis Ltd, receiving fees for acting as chief scientific officer of Phagenesis Ltd, and receiving lecture fees from Nestle

HealthScience. SMG reports reimbursement of travel expenses from Phagenesis Ltd for training and review meetings during the study conduct, and reports fees from Phagenesis Ltd for working in the FEES review board. TW reports reimbursement of travel expenses from Phagenesis for review meetings, and reports that the University Hospital Münster received fees for his work within the FEES review board. PZ reports reimbursement of travel expenses from Phagenesis Ltd for training and review meetings during the study conduct, and reports fees from Phagenesis Ltd for working in the FEES review board. PMB reports personal fees and travel reimbursement from Phagenesis for his role as chair of the scientific committee of PHAST-TRAC, grants from British Heart Foundation, grants from National Institute for Health Research Health Technology Appraisal, share-holding from Platelet Solutions Ltd, personal fees and share-holding from Diamedica, personal fees for working as a consultant from Nestle, Athersys, and Covidien, and personal fees from ReNeuron for chairing a data monitoring committee, and received honorarium and reimbursement of travel expenses from Phagenesis Ltd for his role as co-chief investigator of the Pharyngeal Electrical Stimulation Evaluation for Dysphagia After Stroke (PhEED) trial. PMB also reports that University Hospital Nottingham received payment per patient for study conduct and received payments per patient for the PHADER registry.

Data sharing

The authors will share a subset of anonymised individual patient data with the international VISTA Rehabilitation Collaboration.

Acknowledgments

The trial was sponsored by Phagenesis Ltd.

References

1 Cohen DL, Roffe C, Beavan J, et al. Post-stroke dysphagia: a review and design considerations for future trials. *Int J Stroke* 2016; **11:** 399–411.

2 Smithard DG, Smeeton NC, Wolfe CD. Long-term outcome after stroke: does dysphagia matter? *Age Ageing* 2007; **36:** 90–94.

3 Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke—incidence, diagnosis, and pulmonary complications. *Stroke* 2005; **36:** 2756–63.

4 Woodhouse LJ, Scutt P, Hamdy S, et al. Route of feeding as a proxy for dysphagia after stroke and the effect of transdermal glyceryl trinitrate: data from the efficacy of nitric oxide in stroke randomised controlled trial. *Transl Stroke Res* 2018; **9:** 120–29.

5 Hoffmann S, Malzahn U, Harms H, et al. Development of a clinical score (A2DS2) to predict pneumonia in acute ischemic stroke. *Stroke* 2012; **43:** 2617–23.

6 Foley NC, Martin RE, Salter KL, Teasell RW. A review of the relationship between dysphagia and malnutrition following stroke. *J Rehabil Med* 2009; **41:** 707–13.

7 Walcott BP, Kamel H, Castro B, Kimberly WT, Sheth KN. Tracheostomy after severe ischemic stroke: a population-based study. *J Stroke Cerebrovasc Dis* 2014; **23:** 1024–29.

8 Kurtz P, Fitts V, Sumer Z, et al. How does care differ for neurological patients admitted to a neurocritical care unit versus a general ICU? *Neurocrit Care* 2011; **15:** 477–80.

9 Pelosi P, Ferguson ND, Frutos-Vivar F, et al. Management and outcome of mechanically ventilated neurologic patients. *Crit Care Med* 2011; **39:** 1482–92.

10 Lahiri S, Mayer SA, Fink ME, et al. Mechanical ventilation for acute stroke: a multi-state population-based study. *Neurocrit Care* 2015; **23:** 28–32.

11 Steidl C, Boesel J, Suntrup-Krueger S, et al. Tracheostomy, extubation, reintubation: airway management decisions in intubated stroke patients. *Cerebrovasc Dis* 2017; **44:** 1–9.

12 Bosel J. Use and timing of tracheostomy after severe stroke. *Stroke* 2017; **48:** 2638–43.

13 Lahiri S, Navi BB, Mayer SA, et al. Hospital readmission rates among mechanically ventilated patients with stroke. *Stroke* 2015; **46:** 2969–71.

14 Choate K, Barbetti J, Currey J. Tracheostomy decannulation failure rate following critical illness: a prospective descriptive study. *Aust Crit Care* 2009; **22:** 8–15.

15 Clec'h C, Alberti C, Vincent F, et al. Tracheostomy does not improve the outcome of patients requiring prolonged mechanical ventilation: a propensity analysis. *Crit Care Med* 2007; **35:** 132–38.

16 Martinez GH, Fernandez R, Casado MS, et al. Tracheostomy tube in place at intensive care unit discharge is associated with increased ward mortality. *Respir Care* 2009; **54:** 1644–52.

17 Schneider H, Hertel F, Kuhn M, et al. Decannulation and functional outcome after tracheostomy in patients with severe stroke (DECAST): a prospective observational study. *Neurocrit Care* 2017; **27:** 26–34.

18 Hamdy S, Rothwell JC, Aziz Q, Singh KD, Thompson DG. Long-term reorganization of human motor cortex driven by short-term sensory stimulation. *Nat Neurosci* 1998; **1:** 64–68.

19 Suntrup S, Teismann I, Wollbrink A, et al. Pharyngeal electrical stimulation can modulate swallowing in cortical processing and behavior—magnetoencephalographic evidence. *Neuroimage* 2015; **104:** 117–24.

20 Suntrup-Krueger S, Bittner S, Recker S, et al. Electrical pharyngeal stimulation increases substance P level in saliva. *Neurogastroenterol Motil* 2016; **28:** 855–60.

21 Bath PM, Scutt P, Love J, et al. Pharyngeal electrical stimulation for treatment of dysphagia in subacute stroke: a randomized controlled trial. *Stroke* 2016; **47:** 1562–70.

22 Jayasekeran V, Singh S, Tyrell P, et al. Adjunctive functional pharyngeal electrical stimulation reverses swallowing disability after brain lesions. *Gastroenterol* 2010; **138:** 1737–46.

23 Vasant DH, Michou E, O'Leary N, Vail A, Mistry S, Hamdy S. Pharyngeal electrical stimulation in dysphagia poststroke: a prospective, randomized single-blinded interventional study. *Neurorehabil Neural Repair* 2016; **30:** 866–75.

24 Suntrup S, Marian T, Schroder JB, et al. Electrical pharyngeal stimulation for dysphagia treatment in tracheotomized stroke patients: a randomized controlled trial. *Intensive Care Med* 2015; **41:** 1629–37.

25 Dziewas R, Mistry S, Hamdy S, et al. Design and implementation of Pharyngeal electrical Stimulation for early de-cannulation in TRACheotomized (PHAST-TRAC) stroke patients with neurogenic dysphagia: a prospective randomized single-blinded interventional study. *Int J Stroke* 2017; **12:** 430–37.

26 Whitehead J. The design and analysis of sequential clinical trials, 2nd edn. Chichester: Wiley, 1997.

27 Sessler CN, Gosnell MS, Grap MJ, et al. The Richmond Agitation-Sedation Scale: validity and reliability in adult intensive care unit patients. *Am J Respir Crit Care Med* 2002; **166:** 1338–44.

28 Warnecke T, Suntrup S, Teismann IK, Hamacher C, Oelenberg S, Dziewas R. Standardized endoscopic swallowing evaluation for tracheostomy decannulation in critically ill neurologic patients. *Crit Care Med* 2013; **41:** 1728–32.

29 Crary MA, Mann GD, Groher ME. Initial psychometric assessment of a functional oral intake scale for dysphagia in stroke patients. *Arch Physical Med Rehabil* 2005; **86:** 1516–20.

30 Mehta CR, Patel NR. Exact logistic regression: theory and examples. *Stat Med* 1995; **14:** 2143–60.

31 Medical and Pharmaceutical Statistical Research Unit. PEST 4: operating manual. Reading: The University of Reading, 2000. http://www.mps-research.com/PEST (accessed Aug 13, 2018).

32 R Development Core Team. A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2017.

33 Macht M, Wimbish T, Bodine C, Moss M. ICU-acquired swallowing disorders. *Crit Care Med* 2013; **41:** 2396–405.

34 Jolley SE, Bunnell AE, Hough CL. ICU-acquired weakness. *Chest* 2016; **150:** 1129–40.

35 Muhle P, Suntrup-Krueger S, Bittner S, et al. Increase of Substance P concentration in saliva after pharyngeal electrical stimulation in severely dysphagic stroke patients—an indicator of decannulation success? *Neurosignals* 2017; **25:** 74–87.

For more on the **VISTA Rehabilitation Collaboration** see http://www.vista.gla.ac.uk

6. Abkürzungsverzeichnis

ADC .....*Apparenter Diffusionskoeffizient*
BHS.....*Blut-Hirn-Schranke*
BI..... *Barthel-Index*
CBF.....*Cerebral Blood Flow/Zerebraler Blutfluss*
CBV..... *Cerebral blood Volume/Zerebrales Blutvolumen*
CPG .....*Central Pattern Generator*
CT ..... *Computertomographie*
FEES..... *Flexible Endoskopische Evaluation des Schluckens*
FLAIR.....*Fluid attenuated inversion recovery*
FOIS.....*Functional oral intake scale*
mmSTL ..... *microbubble-mediated sonothrombolysis*
MRT .....*Magnetresonanztomographie*
NPO .....*Nil per os; orale Nahrungskarenz*
PES.....*Pharyngeale Elektrostimulation*
rt-PA.....*Rekombinanter tissue-type Plasminogenaktivator*
STL .....*Sonothrombolyse*
SWI ..... *Susceptibility-weighted imaging*
VFSS..... *Videofluoroscopic Swallowing Study*

7. Literaturverzeichnis

1. Mathers CD, Boerma T, Ma Fat D. Global and regional causes of death. *Br Med Bull*. 2009;92:7-32. doi:10.1093/bmb/ldp028

2. Benjamin EJ, Blaha MJ, Chiuve SE, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation*. 2017;135(10):e146-e603. doi:10.1161/CIR.0000000000000485.

3. Stahmeyer JT, Stubenrauch S, Geyer S, Weissenborn K, Eberhard S. The Frequency and Timing of Recurrent Stroke: An Analysis of Routine Health Insurance Data. *Dtsch Arztebl Int*. 2019;116(42):711-717. doi:10.3238/arztebl.2019.0711

4. Knight-Greenfield A, Nario JJQ, Gupta A. Causes of Acute Stroke: A Patterned Approach. *Radiol Clin North Am*. 2019;57(6):1093-1108. doi:10.1016/j.rcl.2019.07.007

5. Hacke W, ed. *Neurologie.* 14., überarbeitete Auflage ; korrigierte Publikation. Springer; 2019.

6. Heiss WD. Ischemic penumbra: evidence from functional imaging in man. *J Cereb Blood Flow Metab*. 2000;20(9):1276-1293. doi:10.1097/00004647-200009000-00002

7. Hossmann K-A, Traystman RJ. Cerebral blood flow and the ischemic penumbra. *Handb Clin Neurol*. 2009;92:67-92. doi:10.1016/S0072-9752(08)01904-0

8. WEIDNER W, HANAFEEWMARKHAM CH. Intracranial collateral circulation via leptomeningeal and rete mirabile anastomoses. *Neurology*. 1965;15:39-48. doi:10.1212/wnl.15.1.39

9. Liebeskind DS. Collateral circulation. *Stroke*. 2003;34(9):2279-2284. doi:10.1161/01.STR.0000086465.41263.06

10. Siesjö BK. Pathophysiology and treatment of focal cerebral ischemia. Part II: Mechanisms of damage and treatment. *J Neurosurg*. 1992;77(3):337-354. doi:10.3171/jns.1992.77.3.0337

11. Fiebach JB, Al-Rawi Y, Wintermark M, et al. Vascular occlusion enables selecting acute ischemic stroke patients for treatment with desmoteplase. *Stroke*. 2012;43(6):1561-1566. doi:10.1161/STROKEAHA.111.642322

12. Astrup J, Siesjö BK, Symon L. Thresholds in cerebral ischemia - the ischemic penumbra. *Stroke*. 1981;12(6):723-725. doi:10.1161/01.STR.12.6.723

13. Ginsberg MD. Adventures in the pathophysiology of brain ischemia: penumbra, gene expression, neuroprotection: the 2002 Thomas Willis Lecture. *Stroke*. 2003;34(1):214-223. doi:10.1161/01.str.0000048846.09677.62

14. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci*. 1999;22(9):391-397. doi:10.1016/s0166-2236(99)01401-0

15. Rosenberg GA. Ischemic brain edema. *Prog Cardiovasc Dis*. 1999;42(3):209-216. doi:10.1016/s0033-0620(99)70003-4

16. Shaw CM, Alvord EC, Berry RG. Swelling of the brain following ischemic infarction with arterial occlusion. *Arch Neurol*. 1959;1:161-177. doi:10.1001/archneur.1959.03840020035006

17. Dávalos A, Toni D, Iweins F, Lesaffre E, Bastianello S, Castillo J. Neurological deterioration in acute ischemic stroke: potential predictors and associated factors in the European cooperative acute stroke study (ECASS) I. *Stroke*. 1999;30(12):2631-2636. doi:10.1161/01.str.30.12.2631

18. Engelhorn T, Doerfler A, Kastrup A, et al. Decompressive craniectomy, reperfusion, or a combination for early treatment of acute "malignant" cerebral hemispheric stroke in rats? Potential mechanisms studied by MRI. *Stroke*. 1999;30(7):1456-1463. doi:10.1161/01.str.30.7.1456

19. Jüttler E, Schwab S, Schmiedek P, et al. Decompressive Surgery for the Treatment of Malignant Infarction of the Middle Cerebral Artery (DESTINY): a randomized, controlled trial. *Stroke*. 2007;38(9):2518-2525. doi:10.1161/STROKEAHA.107.485649

20. Czosnyka M, Brady K, Reinhard M, Smielewski P, Steiner LA. Monitoring of cerebrovascular autoregulation: facts, myths, and missing links. *Neurocrit Care*. 2009;10(3):373-386. doi:10.1007/s12028-008-9175-7

21. Walberer M, Ritschel N, Nedelmann M, et al. Aggravation of infarct formation by brain swelling in a large territorial stroke: a target for neuroprotection? *J Neurosurg*. 2008;109(2):287-293. doi:10.3171/JNS/2008/109/8/0287

22. Hacke W, Schwab S, Horn M, Spranger M, Georgia M de, Kummer R von. 'Malignant' middle cerebral artery territory infarction: clinical course and prognostic signs. *Arch Neurol*. 1996;53(4):309-315. doi:10.1001/archneur.1996.00550040037012

23. Hossmann K-A. Pathophysiology and therapy of experimental stroke. *Cell Mol Neurobiol*. 2006;26(7-8):1057-1083. doi:10.1007/s10571-006-9008-1

24. Baron JC. Mapping the ischaemic penumbra with PET: implications for acute stroke treatment. *Cerebrovasc Dis*. 1999;9(4):193-201. doi:10.1159/000015955

25. Peter A. Ringleb, Gerhard F.Hamann, Joachim Röther, Olav Jansen, Christoph Groden, Roland Veltkamp. Akuttherapie des ischämischen Schlaganfalls–Ergänzung 2015: Rekanalisierende Therapie. [Leitlinien der Deutschen Gesellschaft für Neurologie]. https://dgn.org/wp-content/uploads/2015/10/030140_LL_akuter-ischaemischer-schlaganfall_final.pdf

26. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med*. 2008;359(13):1317-1329. doi:10.1056/NEJMoa0804656

27. Wahlgren N, Ahmed N, Dávalos A, et al. Thrombolysis with alteplase 3-4.5 h after acute ischaemic stroke (SITS-ISTR): an observational study. *Lancet*. 2008;372(9646):1303-1309. doi:10.1016/S0140-6736(08)61339-2

28. Berkhemer OA, Fransen PSS, Beumer D, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. 2015;372(1):11-20. doi:10.1056/NEJMoa1411587

29. Goyal M, Demchuk AM, Menon BK, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med*. 2015;372(11):1019-1030. doi:10.1056/NEJMoa1414905

30. Campbell BCV, Mitchell PJ, Kleinig TJ, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med*. 2015;372(11):1009-1018. doi:10.1056/NEJMoa1414792

31. Saver JL, Jahan R, Levy EI, et al. Solitaire flow restoration device versus the Merci Retriever in patients with acute ischaemic stroke (SWIFT): a randomised, parallel-group, non-inferiority trial. *Lancet*. 2012;380(9849):1241-1249. doi:10.1016/S0140-6736(12)61384-1

32. Jovin TG, Chamorro A, Cobo E, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med*. 2015;372(24):2296-2306. doi:10.1056/NEJMoa1503780

33. Goyal M, Menon BK, van Zwam WH, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet*. 2016;387(10029):1723-1731. doi:10.1016/S0140-6736(16)00163-X

34. Barow E, Thomalla G. Akutbehandlung des ischämischen Schlaganfalls : Aktuelle Standards. *Nervenarzt*. 2019;90(10):979-986. doi:10.1007/s00115-019-0776-5

35. Organised inpatient (stroke unit) care for stroke. *Cochrane Database Syst Rev*. 2007;(4):CD000197. doi:10.1002/14651858.CD000197.pub2

36. Sobesky J. Akutversorgung des ischämischen Schlaganfalls. *Internist (Berl)*. 2009;50(11):1218-1226. doi:10.1007/s00108-009-2470-3

37. Ahmed N, Wahlgren N, Brainin M, et al. Relationship of blood pressure, antihypertensive therapy, and outcome in ischemic stroke treated with intravenous thrombolysis: retrospective analysis from Safe Implementation of Thrombolysis in Stroke-International Stroke Thrombolysis Register (SITS-ISTR). *Stroke*. 2009;40(7):2442-2449. doi:10.1161/STROKEAHA.109.548602

38. Langhorne P, Pollock A. What are the components of effective stroke unit care? *Age Ageing*. 2002;31(5):365-371. doi:10.1093/ageing/31.5.365

39. Sandercock PAG, Counsell C, Gubitz GJ, Tseng M-C. Antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev*. 2008;(3):CD000029. doi:10.1002/14651858.CD000029.pub2

40. Daniels SK, Huckabee ML, Gozdzikowska K. *Dysphagia Following Stroke.* 3rd ed. Plural Publishing; op. 2019.

41. Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke: incidence, diagnosis, and pulmonary complications. *Stroke*. 2005;36(12):2756-2763. doi:10.1161/01.STR.0000190056.76543.eb

42. Mann G, Hankey GJ, Cameron D. Swallowing function after stroke: prognosis and prognostic factors at 6 months. *Stroke*. 1999;30(4):744-748. doi:10.1161/01.str.30.4.744

43. Holas MA, DePippo KL, Reding MJ. Aspiration and relative risk of medical complications following stroke. *Arch Neurol*. 1994;51(10):1051-1053. doi:10.1001/archneur.1994.00540220099020

44. Kidd D, Lawson J, Nesbitt R, MacMahon J. Aspiration in acute stroke: a clinical study with videofluoroscopy. *Q J Med*. 1993;86(12):825-829.

45. Heuschmann PU, Kolominsky-Rabas PL, Misselwitz B, et al. Predictors of in-hospital mortality and attributable risks of death after ischemic stroke: the German Stroke Registers Study Group. *Arch Intern Med*. 2004;164(16):1761-1768. doi:10.1001/archinte.164.16.1761

46. Scmidt EV, Smirnov VE, Ryabova VS. Results of the seven-year prospective study of stroke patients. *Stroke*. 1988;19(8):942-949. doi:10.1161/01.STR.19.8.942

47. Yoo S-H, Kim JS, Kwon SU, Yun S-C, Koh J-Y, Kang D-W. Undernutrition as a predictor of poor clinical outcomes in acute ischemic stroke patients. *Arch Neurol*. 2008;65(1):39-43. doi:10.1001/archneurol.2007.12

48. Finestone HM, Greene-Finestone LS, Wilson ES, Teasell RW. Malnutrition in stroke patients on the rehabilitation service and at follow-up: prevalence and predictors. *Arch Phys Med Rehabil*. 1995;76(4):310-316.

49. Smithard DG, Smeeton NC, Wolfe CDA. Long-term outcome after stroke: Does dysphagia matter? *Age Ageing*. 2007;36(1):90-94. doi:10.1093/ageing/afl149

50. Warnecke T, Ritter MA, Kroger B, et al. Fiberoptic endoscopic Dysphagia severity scale predicts outcome after acute stroke. *Cerebrovasc Dis*. 2009;28(3):283-289. doi:10.1159/000228711

51. Arnold M, Liesirova K, Broeg-Morvay A, et al. Dysphagia in Acute Stroke: Incidence, Burden and Impact on Clinical Outcome. *PLoS One*. 2016;11(2):e0148424. doi:10.1371/journal.pone.0148424

52. Gonzalez-Fernandez M, Brodsky MB, Palmer JB. Poststroke Communication Disorders and Dysphagia. *Phys Med Rehabil Clin N Am*. 2015;26(4):657-670. doi:10.1016/j.pmr.2015.06.005

53. Bonilha HS, Simpson AN, Ellis C, Mauldin P, Martin-Harris B, Simpson K. The one-year attributable cost of post-stroke dysphagia. *Dysphagia*. 2014;29(5):545-552. doi:10.1007/s00455-014-9543-8

54. Hamdy S, Rothwell JC, Brooks DJ, Bailey D, Aziz Q, Thompson DG. Identification of the cerebral loci processing human swallowing with H2(15)O PET activation. *J Neurophysiol*. 1999;81(4):1917-1926. doi:10.1152/jn.1999.81.4.1917

55. Prosiegel M, Höling R, Heintze M, Wagner-Sonntag E, Wiseman K. The localization of central pattern generators for swallowing in humans--a clinical-anatomical study on patients with unilateral paresis of the vagal nerve, Avellis' syndrome, Wallenberg's syndrome, posterior fossa tumours and cerebellar hemorrhage. *Acta Neurochir Suppl*. 2005;93:85-88. doi:10.1007/3-211-27577-0_13

56. Prosiegel M, Weber S. *Dysphagie: Diagnostik Und Therapie*. *Ein Wegweiser Für Kompetentes Handeln.* 3. Auflage 2018. Springer Berlin Heidelberg; 2018.

57. Leopold NA, Daniels SK. Supranuclear control of swallowing. *Dysphagia*. 2010;25(3):250-257. doi:10.1007/s00455-009-9249-5

58. Saur D, Lange R, Baumgaertner A, et al. Dynamics of language reorganization after stroke. *Brain*. 2006;129(Pt 6):1371-1384. doi:10.1093/brain/awl090

59. Jean A. Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev*. 2001;81(2):929-969. doi:10.1152/physrev.2001.81.2.929

60. Warnecke T, Dziewas R. *Neurogene Dysphagien: Diagnostik Und Therapie.* 2., erweiterte und überarbeite Auflage. Verlag W. Kohlhammer; 2018.

61. Langmore SE, Schatz K, Olsen N. Fiberoptic endoscopic examination of swallowing safety: a new procedure. *Dysphagia*. 1988;2(4):216-219. doi:10.1007/BF02414429

62. Langmore SE. *Endoscopic Evaluation and Treatment of Swallowing Disorders.* Second edition. Thieme; 2001.

63. Flader CM, Rosendahl C, Günther T. Leitlinienkonforme Dysphagiediagnostik : Eine repräsentative Befragung von Logopäden an zertifizierten Stroke-Units. *Nervenarzt*. 2017;88(10):1168-1176. doi:10.1007/s00115-017-0322-2

64. Steele CM, Alsanei WA, Ayanikalath S, et al. The influence of food texture and liquid consistency modification on swallowing physiology and function: a systematic review. *Dysphagia*. 2015;30(1):2-26. doi:10.1007/s00455-014-9578-x

65. Newman R, Vilardell N, Clavé P, Speyer R. Effect of Bolus Viscosity on the Safety and Efficacy of Swallowing and the Kinematics of the Swallow Response in Patients with Oropharyngeal Dysphagia: White Paper by the European Society for Swallowing Disorders (ESSD). *Dysphagia*. 2016;31(2):232-249. doi:10.1007/s00455-016-9696-8

66. Nakagawa T, Wada H, Sekizawa K, Arai H, Sasaki H. Amantadine and pneumonia. *Lancet*. 1999;353(9159):1157. doi:10.1016/S0140-6736(98)05805-X

67. Ebihara S, Ebihara T, Gui P, Osaka K, Sumi Y, Kohzuki M. Thermal taste and anti-aspiration drugs: a novel drug discovery against pneumonia. *Curr Pharm Des*. 2014;20(16):2755-2759. doi:10.2174/13816128113199990567

68. Ohkubo T, Chapman N, Neal B, Woodward M, Omae T, Chalmers J. Effects of an angiotensin-converting enzyme inhibitor-based regimen on pneumonia risk. *Am J Respir Crit Care Med*. 2004;169(9):1041-1045. doi:10.1164/rccm.200309-1219OC

69. Chiu M-J, Chang Y-C, Hsiao T-Y. Prolonged effect of botulinum toxin injection in the treatment of cricopharyngeal dysphagia: case report and literature review. *Dysphagia*. 2004;19(1):52-57. doi:10.1007/s00455-003-0029-3

70. Gallas S, Marie JP, Leroi AM, Verin E. Sensory transcutaneous electrical stimulation improves post-stroke dysphagic patients. *Dysphagia*. 2010;25(4):291-297. doi:10.1007/s00455-009-9259-3

71. Mistry S, Verin E, Singh S, et al. Unilateral suppression of pharyngeal motor cortex to repetitive transcranial magnetic stimulation reveals functional asymmetry in the hemispheric

projections to human swallowing. *J Physiol*. 2007;585(Pt 2):525-538. doi:10.1113/jphysiol.2007.144592

72. Kumar S, Wagner CW, Frayne C, et al. Noninvasive brain stimulation may improve stroke-related dysphagia: a pilot study. *Stroke*. 2011;42(4):1035-1040. doi:10.1161/STROKEAHA.110.602128

73. Fraser C, Power M, Hamdy S, et al. Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. *Neuron*. 2002;34(5):831-840. doi:10.1016/s0896-6273(02)00705-5

74. Hamdy S, Rothwell JC, Aziz Q, Singh KD, Thompson DG. Long-term reorganization of human motor cortex driven by short-term sensory stimulation. *Nat Neurosci*. 1998;1(1):64-68. doi:10.1038/264

75. Suntrup S, Teismann I, Wollbrink A, et al. Pharyngeal electrical stimulation can modulate swallowing in cortical processing and behavior - magnetoencephalographic evidence. *Neuroimage*. 2015;104:117-124. doi:10.1016/j.neuroimage.2014.10.016

76. Suntrup-Krueger S, Bittner S, Recker S, et al. Electrical pharyngeal stimulation increases substance P level in saliva. *Neurogastroenterol Motil*. 2016;28(6):855-860. doi:10.1111/nmo.12783

77. Bath PM, Scutt P, Love J, et al. Pharyngeal Electrical Stimulation for Treatment of Dysphagia in Subacute Stroke: A Randomized Controlled Trial. *Stroke*. 2016;47(6):1562-1570. doi:10.1161/STROKEAHA.115.012455

78. Richter D, Eyding J, Weber R, et al. Analysis of Nationwide Stroke Patient Care in Times of COVID-19 Pandemic in Germany. *Stroke*. 2021;52(2):716-721. doi:10.1161/STROKEAHA.120.033160.

79. Hacke W, Donnan G, Fieschi C, et al. Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. *Lancet*. 2004;363(9411):768-774. doi:10.1016/S0140-6736(04)15692-4

80. Mori E, Yoneda Y, Tabuchi M, et al. Intravenous recombinant tissue plasminogen activator in acute carotid artery territory stroke. *Neurology*. 1992;42(5):976-982. doi:10.1212/wnl.42.5.976

81. Rha J-H, Saver JL. The impact of recanalization on ischemic stroke outcome: a meta-analysis. *Stroke*. 2007;38(3):967-973. doi:10.1161/01.STR.0000258112.14918.24.

82. Tsivgoulis G, Katsanos AH, Schellinger PD, et al. Successful Reperfusion With Intravenous Thrombolysis Preceding Mechanical Thrombectomy in Large-Vessel Occlusions. *Stroke*. 2018;49(1):232-235. doi:10.1161/STROKEAHA.117.019261

83. Heldner MR, Zubler C, Mattle HP, et al. National Institutes of Health stroke scale score and vessel occlusion in 2152 patients with acute ischemic stroke. *Stroke*. 2013;44(4):1153-1157. doi:10.1161/STROKEAHA.111.000604

84. Beumer D, Mulder MJHL, Saiedie G, et al. Occurrence of intracranial large vessel occlusion in consecutive, non-referred patients with acute ischemic stroke. *Neurovasc Imaging*. 2016;2(1). doi:10.1186/s40809-016-0022-5

85. Rabinstein AA. Treatment of Acute Ischemic Stroke. *Continuum (Minneap Minn)*. 2017;23(1, Cerebrovascular Disease):62-81. doi:10.1212/CON.0000000000000420.

86. Kahle MP, Bix GJ. Successfully Climbing the "STAIRs": Surmounting Failed Translation of Experimental Ischemic Stroke Treatments. *Stroke Res Treat*. 2012;2012:374098. doi:10.1155/2012/374098

87. Yoo AJ, Chaudhry ZA, Nogueira RG, et al. Infarct volume is a pivotal biomarker after intra-arterial stroke therapy. *Stroke*. 2012;43(5):1323-1330. doi:10.1161/STROKEAHA.111.639401

88. Edgell RC, Vora NA. Neuroimaging markers of hemorrhagic risk with stroke reperfusion therapy. *Neurology*. 2012;79(13 Suppl 1):S100-4. doi:10.1212/WNL.0b013e3182695848

89. Acer N, Sahin B, Usanmaz M, Tatoğlu H, Irmak Z. Comparison of point counting and planimetry methods for the assessment of cerebellar volume in human using magnetic resonance imaging: a stereological study. *Surg Radiol Anat*. 2008;30(4):335-339. doi:10.1007/s00276-008-0330-9

90. Siddiqi F, Blinc A, Braaten J, Francis CW. Ultrasound increases flow through fibrin gels. *Thromb Haemost*. 1995;73(3):495-498.

91. Wang Z, Fukuda T, Azuma T, Furuhata H. Safety of low-frequency transcranial ultrasound in permanent middle cerebral artery occlusion in spontaneously hypertensive rats. *Cerebrovasc Dis*. 2012;33(1):23-29. doi:10.1159/000332034

92. Akiyama M, Ishibashi T, Yamada T, Furuhata H. Low-frequency ultrasound penetrates the cranium and enhances thrombolysis in vitro. *Neurosurgery*. 1998;43(4):828-32; discussion 832-3. doi:10.1097/00006123-199810000-00062

93. Alexandrov AV, Molina CA, Grotta JC, et al. Ultrasound-enhanced systemic thrombolysis for acute ischemic stroke. *N Engl J Med*. 2004;351(21):2170-2178. doi:10.1056/NEJMoa041175

94. Eggers J, Koch B, Meyer K, König I, Seidel G. Effect of ultrasound on thrombolysis of middle cerebral artery occlusion. *Ann Neurol*. 2003;53(6):797-800. doi:10.1002/ana.10590

95. Datta S, Coussios C-C, Ammi AY, Mast TD, Courten-Myers GM de, Holland CK. Ultrasound-enhanced thrombolysis using Definity as a cavitation nucleation agent. *Ultrasound Med Biol*. 2008;34(9):1421-1433. doi:10.1016/j.ultrasmedbio.2008.01.016

96. Nedelmann M, Ritschel N, Doenges S, et al. Combined contrast-enhanced ultrasound and rt-PA treatment is safe and improves impaired microcirculation after reperfusion of middle cerebral artery occlusion. *J Cereb Blood Flow Metab*. 2010;30(10):1712-1720. doi:10.1038/jcbfm.2010.82

97. Block F, Schwarz M. Neuroprotektion beim Schlaganfall. Eine kritische Bestandsaufnahme. *Nervenarzt*. 1999;70(2):101-110. doi:10.1007/s001150050409

98. Neuhaus AA, Couch Y, Hadley G, Buchan AM. Neuroprotection in stroke: the importance of collaboration and reproducibility. *Brain*. 2017;140(8):2079-2092. doi:10.1093/brain/awx126

99. Walberer M, Blaes F, Stolz E, et al. Midline-shift corresponds to the amount of brain edema early after hemispheric stroke--an MRI study in rats. *J Neurosurg Anesthesiol*. 2007;19(2):105-110. doi:10.1097/ANA.0b013e31802c7e33

100. Gerriets T, Stolz E, Walberer M, et al. Noninvasive quantification of brain edema and the space-occupying effect in rat stroke models using magnetic resonance imaging. *Stroke*. 2004;35(2):566-571. doi:10.1161/01.STR.0000113692.38574.57

101. Gerriets T, Stolz E, Walberer M, et al. Middle cerebral artery occlusion during MR-imaging: investigation of the hyperacute phase of stroke using a new in-bore occlusion model in rats. *Brain Res Brain Res Protoc*. 2004;12(3):137-143. doi:10.1016/j.brainresprot.2003.08.006

102. Walberer M, Tschernatsch M, Fischer S, et al. RNase therapy assessed by magnetic resonance imaging reduces cerebral edema and infarction size in acute stroke. *Curr Neurovasc Res*. 2009;6(1):12-19. doi:10.2174/156720209787466037

103. Vahedi K, Hofmeijer J, Juettler E, et al. Early decompressive surgery in malignant infarction of the middle cerebral artery: a pooled analysis of three randomised controlled trials. *The Lancet Neurology*. 2007;6(3):215-222. doi:10.1016/S1474-4422(07)70036-4

104. Schwab S, Steiner T, Aschoff A, et al. Early hemicraniectomy in patients with complete middle cerebral artery infarction. *Stroke*. 1998;29(9):1888-1893. doi:10.1161/01.str.29.9.1888

105. Engelhorn T, Kummer R von, Reith W, Forsting M, Doerfler A. What is effective in malignant middle cerebral artery infarction: reperfusion, craniectomy, or both? An experimental study in rats. *Stroke*. 2002;33(2):617-622. doi:10.1161/hs0202.102374

106. Hofmeijer J, Schepers J, Veldhuis WB, et al. Delayed decompressive surgery increases apparent diffusion coefficient and improves peri-infarct perfusion in rats with space-occupying cerebral infarction. *Stroke*. 2004;35(6):1476-1481. doi:10.1161/01.STR.0000128415.31274.3a

107. Ratilal BO, Arroja MMC, Rocha JPF, et al. Neuroprotective effects of erythropoietin pretreatment in a rodent model of transient middle cerebral artery occlusion. *J Neurosurg*. 2014;121(1):55-62. doi:10.3171/2014.2.JNS132197

108. Bath PM, Lee HS, Everton LF. Swallowing therapy for dysphagia in acute and subacute stroke. *Cochrane Database Syst Rev*. 2018;10:CD000323. doi:10.1002/14651858.CD000323.pub3

109. Crary MA, Mann GDC, Groher ME. Initial psychometric assessment of a functional oral intake scale for dysphagia in stroke patients. *Arch Phys Med Rehabil*. 2005;86(8):1516-1520. doi:10.1016/j.apmr.2004.11.049

110. Cruz-Orive LM. Stereology of single objects. *Journal of Microscopy*. 1997;186(2):93-107. doi:10.1046/j.1365-2818.1997.1380695.x

111. Wada A, Kawakami M, Liu M, et al. Development of a new scale for dysphagia in patients with progressive neuromuscular diseases: the Neuromuscular Disease Swallowing Status Scale (NdSSS). *J Neurol*. 2015;262(10):2225-2231. doi:10.1007/s00415-015-7836-y

112. Christiaanse ME, Mabe B, Russell G, Simeone TL, Fortunato J, Rubin B. Neuromuscular electrical stimulation is no more effective than usual care for the treatment of primary dysphagia in children. *Pediatr Pulmonol*. 2011;46(6):559-565. doi:10.1002/ppul.21400

113. Bhatt AD, Goodwin N, Cash E, et al. Impact of transcutaneous neuromuscular electrical stimulation on dysphagia in patients with head and neck cancer treated with definitive chemoradiation. *Head Neck*. 2015;37(7):1051-1056. doi:10.1002/hed.23708

114. Heijnen BJ, Speyer R, Baijens LWJ, Bogaardt HCA. Neuromuscular electrical stimulation versus traditional therapy in patients with Parkinson's disease and oropharyngeal dysphagia: effects on quality of life. *Dysphagia*. 2012;27(3):336-345. doi:10.1007/s00455-011-9371-z

115. Salassa JR. A functional outcome swallowing scale for staging oropharyngeal dysphagia. *Dig Dis*. 1999;17(4):230-234. doi:10.1159/000016941

116. Kunieda K, Ohno T, Fujishima I, Hojo K, Morita T. Reliability and validity of a tool to measure the severity of dysphagia: the Food Intake LEVEL Scale. *J Pain Symptom Manage*. 2013;46(2):201-206. doi:10.1016/j.jpainsymman.2012.07.020

117. O'Neil KH, Purdy M, Falk J, Gallo L. The Dysphagia Outcome and Severity Scale. *Dysphagia*. 1999;14(3):139-145. doi:10.1007/PL00009595

118. Dziewas R, Warnecke T, Olenberg S, et al. Towards a basic endoscopic assessment of swallowing in acute stroke - development and evaluation of a simple dysphagia score. *Cerebrovasc Dis*. 2008;26(1):41-47. doi:10.1159/000135652

119. Macht M, Wimbish T, Bodine C, Moss M. ICU-acquired swallowing disorders. *Crit Care Med*. 2013;41(10):2396-2405. doi:10.1097/CCM.0b013e31829caf33

120. Puthucheary Z, Harridge S, Hart N. Skeletal muscle dysfunction in critical care: wasting, weakness, and rehabilitation strategies. *Crit Care Med*. 2010;38(10 Suppl):S676-82. doi:10.1097/CCM.0b013e3181f2458d

121. Butterworth CE. The skeleton in the hospital closet. *Nutrition*. 1994;10(5):442.

122. Souza TT, Sturion CJ, Faintuch J. Is the skeleton still in the hospital closet? A review of hospital malnutrition emphasizing health economic aspects. *Clin Nutr*. 2015;34(6):1088-1092. doi:10.1016/j.clnu.2015.02.008

123. Schumann B, Büßelberg N, Stanschus S, Willmes K, Andres F, Binkofski F. Risikofaktoren für Pneumonie bei akuten Schlaganfallpatienten mit Dysphagie. *Sprache Stimme Gehör*. 2012;36(S 01):e20-e21. doi:10.1055/s-0032-1304897

124. Newman AB, Yanez D, Harris T, Duxbury A, Enright PL, Fried LP. Weight change in old age and its association with mortality. *J Am Geriatr Soc*. 2001;49(10):1309-1318. doi:10.1046/j.1532-5415.2001.49258.x

125. El Solh A, Okada M, Bhat A, Pietrantoni C. Swallowing disorders post orotracheal intubation in the elderly. *Intensive Care Med*. 2003;29(9):1451-1455. doi:10.1007/s00134-003-1870-4

126. Hafner G, Neuhuber A, Hirtenfelder S, Schmedler B, Eckel HE. Fiberoptic endoscopic evaluation of swallowing in intensive care unit patients. *Eur Arch Otorhinolaryngol*. 2008;265(4):441-446. doi:10.1007/s00405-007-0507-6

127. Walcott BP, Kamel H, Castro B, Kimberly WT, Sheth KN. Tracheostomy after severe ischemic stroke: a population-based study. *J Stroke Cerebrovasc Dis*. 2014;23(5):1024-1029. doi:10.1016/j.jstrokecerebrovasdis.2013.08.019

128. Kurtz P, Fitts V, Sumer Z, et al. How does care differ for neurological patients admitted to a neurocritical care unit versus a general ICU? *Neurocrit Care*. 2011;15(3):477-480. doi:10.1007/s12028-011-9539-2

129. Pelosi P, Ferguson ND, Frutos-Vivar F, et al. Management and outcome of mechanically ventilated neurologic patients. *Crit Care Med*. 2011;39(6):1482-1492. doi:10.1097/CCM.0b013e31821209a8

130. Lahiri S, Mayer SA, Fink ME, et al. Mechanical Ventilation for Acute Stroke: A Multi-state Population-Based Study. *Neurocrit Care*. 2015;23(1):28-32. doi:10.1007/s12028-014-0082-9

131. Bösel J. Use and Timing of Tracheostomy After Severe Stroke. *Stroke*. 2017;48(9):2638-2643. doi:10.1161/STROKEAHA.117.017794

132. Lahiri S, Navi BB, Mayer SA, et al. Hospital Readmission Rates Among Mechanically Ventilated Patients With Stroke. *Stroke*. 2015;46(10):2969-2971. doi:10.1161/STROKEAHA.115.010441

133. Choate K, Barbetti J, Currey J. Tracheostomy decannulation failure rate following critical illness: a prospective descriptive study. *Aust Crit Care*. 2009;22(1):8-15. doi:10.1016/j.aucc.2008.10.002

134. Clec'h C, Alberti C, Vincent F, et al. Tracheostomy does not improve the outcome of patients requiring prolonged mechanical ventilation: a propensity analysis. *Crit Care Med*. 2007;35(1):132-138. doi:10.1097/01.CCM.0000251134.96055.A6

135. Martinez GH, Fernandez R, Casado MS, et al. Tracheostomy tube in place at intensive care unit discharge is associated with increased ward mortality. *Respir Care*. 2009;54(12):1644-1652.

136. Schneider H, Hertel F, Kuhn M, et al. Decannulation and Functional Outcome After Tracheostomy in Patients with Severe Stroke (DECAST): A Prospective Observational Study. *Neurocrit Care*. 2017;27(1):26-34. doi:10.1007/s12028-017-0390-y

137. Cohen DL, Roffe C, Beavan J, et al. Post-stroke dysphagia: A review and design considerations for future trials. *Int J Stroke*. 2016;11(4):399-411. doi:10.1177/1747493016639057

138. Suntrup S, Marian T, Schröder JB, et al. Electrical pharyngeal stimulation for dysphagia treatment in tracheotomized stroke patients: a randomized controlled trial. *Intensive Care Med*. 2015;41(9):1629-1637. doi:10.1007/s00134-015-3897-8

139. Jüttler E, Unterberg A, Woitzik J, et al. Hemicraniectomy in older patients with extensive middle-cerebral-artery stroke. *N Engl J Med*. 2014;370(12):1091-1100. doi:10.1056/NEJMoa1311367

140. Langhorne P, Ramachandra S. Organised inpatient (stroke unit) care for stroke: network meta-analysis. *Cochrane Database Syst Rev*. 2020;4:CD000197. doi:10.1002/14651858.CD000197.pub4.

141. Sandercock PAG, Counsell C, Tseng M-C, Cecconi E. Oral antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev*. 2014;(3):CD000029. doi:10.1002/14651858.CD000029.pub3.

142. Nagel S, Wagner S, Koziol J, Kluge B, Heiland S. Volumetric evaluation of the ischemic lesion size with serial MRI in a transient MCAO model of the rat: comparison of DWI and T1WI. *Brain Res Brain Res Protoc*. 2004;12(3):172-179. doi:10.1016/j.brainresprot.2003.11.004

143. Friedländer F, Bohmann F, Brunkhorst M, et al. Reliability of infarct volumetry: Its relevance and the improvement by a software-assisted approach. *J Cereb Blood Flow Metab*. 2017;37(8):3015-3026. doi:10.1177/0271678X16681311

144. Sims JR, Gharai LR, Schaefer PW, et al. ABC/2 for rapid clinical estimate of infarct, perfusion, and mismatch volumes. *Neurology*. 2009;72(24):2104-2110. doi:10.1212/WNL.0b013e3181aa5329

145. Meng X, Fisher M, Shen Q, Sotak CH, Duong TQ. Characterizing the diffusion/perfusion mismatch in experimental focal cerebral ischemia. *Ann Neurol*. 2004;55(2):207-212. doi:10.1002/ana.10803

146. Molina CA, Montaner J, Abilleira S, et al. Timing of spontaneous recanalization and risk of hemorrhagic transformation in acute cardioembolic stroke. *Stroke*. 2001;32(5):1079-1084. doi:10.1161/01.str.32.5.1079

147. Auboire L, Sennoga CA, Hyvelin J-M, et al. Microbubbles combined with ultrasound therapy in ischemic stroke: A systematic review of in-vivo preclinical studies. *PLoS One*. 2018;13(2):e0191788. doi:10.1371/journal.pone.0191788

148. Schleicher N, Tomkins AJ, Kampschulte M, et al. Sonothrombolysis with BR38 Microbubbles Improves Microvascular Patency in a Rat Model of Stroke. *PLoS One*. 2016;11(4):e0152898. doi:10.1371/journal.pone.0152898

149. Tomkins AJ, Schleicher N, Murtha L, et al. Platelet rich clots are resistant to lysis by thrombolytic therapy in a rat model of embolic stroke. *Exp Transl Stroke Med*. 2015;7:2. doi:10.1186/s13231-014-0014-y

150. Ren X, Wang Y, Wang Y, et al. Thrombolytic therapy with rt-PA and transcranial color Doppler ultrasound (TCCS) combined with microbubbles for embolic thrombus. *Thromb Res*. 2015;136(5):1027-1032. doi:10.1016/j.thromres.2015.08.027

151. Moumouh A, Barentin L, Tranquart F, Serrierre S, Bonnaud I, Tasu JP. Fibrinolytic effects of transparietal ultrasound associated with intravenous infusion of an ultrasound contrast agent: study of a rat model of acute cerebral stroke. *Ultrasound Med Biol*. 2010;36(1):51-57. doi:10.1016/j.ultrasmedbio.2009.06.1103

152. Lu Y, Wang J, Huang R, et al. Microbubble-Mediated Sonothrombolysis Improves Outcome After Thrombotic Microembolism-Induced Acute Ischemic Stroke. *Stroke*. 2016;47(5):1344-1353. doi:10.1161/STROKEAHA.115.012056

153. Dixon AJ, Li J, Rickel J-MR, Klibanov AL, Zuo Z, Hossack JA. Efficacy of Sonothrombolysis Using Microbubbles Produced by a Catheter-Based Microfluidic Device in a Rat Model of Ischemic Stroke. *Ann Biomed Eng*. 2019;47(4):1012-1022. doi:10.1007/s10439-019-02209-0

154. Alonso A, Dempfle C-E, Della Martina A, et al. In vivo clot lysis of human thrombus with intravenous abciximab immunobubbles and ultrasound. *Thromb Res*. 2009;124(1):70-74. doi:10.1016/j.thromres.2008.11.019

155. Brown AT, Flores R, Hamilton E, Roberson PK, Borrelli MJ, Culp WC. Microbubbles improve sonothrombolysis in vitro and decrease hemorrhage in vivo in a rabbit stroke model. *Invest Radiol*. 2011;46(3):202-207. doi:10.1097/RLI.0b013e318200757a

156. Bader KB, Gruber MJ, Holland CK. Shaken and stirred: mechanisms of ultrasound-enhanced thrombolysis. *Ultrasound Med Biol*. 2015;41(1):187-196. doi:10.1016/j.ultrasmedbio.2014.08.018

157. Bernaudin M, Marti HH, Roussel S, et al. A potential role for erythropoietin in focal permanent cerebral ischemia in mice. *J Cereb Blood Flow Metab*. 1999;19(6):643-651. doi:10.1097/00004647-199906000-00007

158. Dahlberg SA, Xu L, Hess DC, Hohnadel E, Hill WD, Fagan SC. Erythropoietin and Erythropoietin Mimetic Peptide in Focal Cerebral Ischemia. *Stroke*. 2004;35(1):279. doi:10.1161/str.35.1.253

159. Wang R, Wu X, Liang J, et al. Intra-artery infusion of recombinant human erythropoietin reduces blood-brain barrier disruption in rats following cerebral ischemia and reperfusion. *Int J Neurosci*. 2015;125(9):693-702. doi:10.3109/00207454.2014.966354

160. Chi OZ, Hunter C, Liu X, Weiss HR. Effects of erythropoietin on blood-brain barrier disruption in focal cerebral ischemia. *Pharmacology*. 2008;82(1):38-42. doi:10.1159/000127839

161. Zechariah A, ElAli A, Hermann DM. Combination of tissue-plasminogen activator with erythropoietin induces blood-brain barrier permeability, extracellular matrix disaggregation, and DNA fragmentation after focal cerebral ischemia in mice. *Stroke*. 2010;41(5):1008-1012. doi:10.1161/STROKEAHA.109.574418

162. Li Y, Lu Z-Y, Ogle M, Wei L. Erythropoietin prevents blood brain barrier damage induced by focal cerebral ischemia in mice. *Neurochem Res*. 2007;32(12):2132-2141. doi:10.1007/s11064-007-9387-9

163. Gunnarson E, Song Y, Kowalewski JM, et al. Erythropoietin modulation of astrocyte water permeability as a component of neuroprotection. *Proc Natl Acad Sci U S A*. 2009;106(5):1602-1607. doi:10.1073/pnas.0812708106

164. Calapai G, Marciano MC, Corica F, et al. Erythropoietin protects against brain ischemic injury by inhibition of nitric oxide formation. *Eur J Pharmacol*. 2000;401(3):349-356. doi:10.1016/s0014-2999(00)00466-0

165. Blasig IE, Mertsch K, Haseloff RF. Nitronyl nitroxides, a novel group of protective agents against oxidative stress in endothelial cells forming the blood-brain barrier. *Neuropharmacology*. 2002;43(6):1006-1014. doi:10.1016/s0028-3908(02)00180-6

166. Bahcekapili N, Uzüm G, Gökkusu C, Kuru A, Ziylan YZ. The relationship between erythropoietin pretreatment with blood-brain barrier and lipid peroxidation after ischemia/reperfusion in rats. *Life Sci*. 2007;80(14):1245-1251. doi:10.1016/j.lfs.2006.12.014

167. Utepbergenov DI, Mertsch K, Sporbert A, et al. Nitric oxide protects blood-brain barrier in vitro from hypoxia/reoxygenation-mediated injury. *FEBS Lett*. 1998;424(3):197-201. doi:10.1016/s0014-5793(98)00173-2

168. Juenemann M, Goegel S, Obert M, et al. Flat-panel volumetric computed tomography in cerebral perfusion: evaluation of three rat stroke models. *J Neurosci Methods*. 2013;219(1):113-123. doi:10.1016/j.jneumeth.2013.07.010

169. Xiong Y, Mahmood A, Qu C, et al. Erythropoietin improves histological and functional outcomes after traumatic brain injury in mice in the absence of the neural erythropoietin receptor. *J Neurotrauma*. 2010;27(1):205-215. doi:10.1089/neu.2009.1001

170. Li Y, Lu Z, Keogh CL, Yu SP, Wei L. Erythropoietin-induced neurovascular protection, angiogenesis, and cerebral blood flow restoration after focal ischemia in mice. *J Cereb Blood Flow Metab*. 2007;27(5):1043-1054. doi:10.1038/sj.jcbfm.9600417

171. Murphy AM, Xenocostas A, Pakkiri P, Lee T-Y. Hemodynamic effects of recombinant human erythropoietin on the central nervous system after subarachnoid hemorrhage: reduction of microcirculatory impairment and functional deficits in a rabbit model. *J Neurosurg*. 2008;109(6):1155-1164. doi:10.3171/JNS.2008.109.12.1155

172. Shafi NI, Andresen J, Marrelli SP, Bryan RM. Erythropoietin potentiates EDHF-mediated dilations in rat middle cerebral arteries. *J Neurotrauma*. 2008;25(3):257-265. doi:10.1089/neu.2007.0347

173. Leist M, Ghezzi P, Grasso G, et al. Derivatives of erythropoietin that are tissue protective but not erythropoietic. *Science*. 2004;305(5681):239-242. doi:10.1126/science.1098313

174. Zhang F, Xing J, Liou AK-F, et al. Enhanced Delivery of Erythropoietin Across the Blood-Brain Barrier for Neuroprotection against Ischemic Neuronal Injury. *Transl Stroke Res*. 2010;1(2):113-121. doi:10.1007/s12975-010-0019-3.

175. Lieutaud T, Andrews PJD, Rhodes JKJ, Williamson R. Characterization of the pharmacokinetics of human recombinant erythropoietin in blood and brain when administered immediately after lateral fluid percussion brain injury and its pharmacodynamic effects on IL-1beta and MIP-2 in rats. *J Neurotrauma*. 2008;25(10):1179-1185. doi:10.1089/neu.2008.0591

176. Xenocostas A, Cheung WK, Farrell F, et al. The pharmacokinetics of erythropoietin in the cerebrospinal fluid after intravenous administration of recombinant human erythropoietin. *Eur J Clin Pharmacol*. 2005;61(3):189-195. doi:10.1007/s00228-005-0896-7

177. Schmitt M, Eid M. Richtlinien für die Übersetzung fremdsprachlicher Messinstrumente. *Diagnostica*. 2007;53(1):1-2. doi:10.1026/0012-1924.53.1.1

178. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. *Spine (Phila Pa 1976)*. 2000;25(24):3186-3191. doi:10.1097/00007632-200012150-00014

179. Behr D. *Translationswissenschaft und international vergleichende Umfrageforschung: Qualitätssicherung bei Fragebogenübersetzungen als Gegenstand einer Prozessanalyse*: SSOAR - GESIS Leibniz Institute for the Social Sciences; 2009.

180. Guillemin F, Bombardier C, Beaton D. Cross-cultural adaptation of health-related quality of life measures: Literature review and proposed guidelines. *Journal of Clinical Epidemiology*. 1993;46(12):1417-1432. doi:10.1016/0895-4356(93)90142-N

181. Zhou Hongzhen, Zhu Yafang, Zhang Xiaomei. Validation of the Chinese Version of the Functional Oral Intake Scale (FOIS) Score in the Assessment of Acute Stroke Patients with Dysphagia. *Studies in Health Technology and Informatics*. 2017;245:1195-1199. doi:10.3233/978-1-61499-830-3-1195

182. Battel I, Calvo I, Walshe M. Cross-Cultural Validation of the Italian Version of the Functional Oral Intake Scale. *Folia Phoniatr Logop*. 2018;70(3-4):117-123. doi:10.1159/000490792

183. Trapl M, Enderle P, Nowotny M, et al. Dysphagia bedside screening for acute-stroke patients: The Gugging Swallowing Screen. *Stroke*. 2007;38(11):2948-2952. doi:10.1161/STROKEAHA.107.483933

184. Teismann IK, Dziewas R, Steinstraeter O, Pantev C. Time-dependent hemispheric shift of the cortical control of volitional swallowing. *Hum Brain Mapp*. 2009;30(1):92-100. doi:10.1002/hbm.20488

185. Andre JM, Beis JM, Morin N, Paysant J. Buccal hemineglect. *Arch Neurol*. 2000;57(12):1734-1741.

186. Parker C, Power M, Hamdy S, Bowen A, Tyrrell P, Thompson DG. Awareness of dysphagia by patients following stroke predicts swallowing performance. *Dysphagia*. 2004;19(1):28-35. doi:10.1007/s00455-003-0032-8

187. Lindner-Pfleghar B, Neugebauer H, Stosser S, et al. Dysphagiemanagement beim akuten Schlaganfall: Eine prospektive Studie zur Uberprufung der geltenden Empfehlungen. *Nervenarzt*. 2017;88(2):173-179. doi:10.1007/s00115-016-0271-1

188. Dziewas R, dem Brinke M auf, Birkmann U, et al. Safety and clinical impact of FEES – results of the FEES-registry. *Neurol Res Pract*. 2019;1(1):2756. doi:10.1186/s42466-019-0021-5

189. Bax L, McFarlane M, Green E, Miles A. Speech-language pathologist-led fiberoptic endoscopic evaluation of swallowing: functional outcomes for patients after stroke. *J Stroke Cerebrovasc Dis*. 2014;23(3):e195-200. doi:10.1016/j.jstrokecerebrovasdis.2013.09.031

190. Nguile-Makao M, Zahar J-R, Français A, et al. Attributable mortality of ventilator-associated pneumonia: respective impact of main characteristics at ICU admission and VAP onset using conditional logistic regression and multi-state models. *Intensive Care Med*. 2010;36(5):781-789. doi:10.1007/s00134-010-1824-6

191. Rotstein C, Evans G, Born A, et al. Clinical practice guidelines for hospital-acquired pneumonia and ventilator-associated pneumonia in adults. *Can J Infect Dis Med Microbiol*. 2008;19(1):19-53. doi:10.1155/2008/593289

192. Martin-Harris B, Logemann JA, McMahon S, Schleicher M, Sandidge J. Clinical utility of the modified barium swallow. *Dysphagia*. 2000;15(3):136-141. doi:10.1007/s004550010015

193. Hales PA, Drinnan MJ, Wilson JA. The added value of fibreoptic endoscopic evaluation of swallowing in tracheostomy weaning. *Clin Otolaryngol*. 2008;33(4):319-324. doi:10.1111/j.1749-4486.2008.01757.x

194. McCullough GH, Wertz RT, Rosenbek JC. Sensitivity and specificity of clinical/bedside examination signs for detecting aspiration in adults subsequent to stroke. *J Commun Disord*. 2001;34(1-2):55-72. doi:10.1016/S0021-9924(00)00041-1

195. Lynch YT, Clark BJ, Macht M, et al. The accuracy of the bedside swallowing evaluation for detecting aspiration in survivors of acute respiratory failure. *J Crit Care*. 2017;39:143-148. doi:10.1016/j.jcrc.2017.02.013

196. Chapman C, Morgan P, Cadilhac DA, Purvis T, Andrew NE. Risk factors for the development of chest infections in acute stroke: a systematic review. *Top Stroke Rehabil*. 2018;25(6):445-458. doi:10.1080/10749357.2018.1481567

197. Wästfelt M, Cao Y, Ström JO. Predictors of post-stroke fever and infections: a systematic review and meta-analysis. *BMC Neurol*. 2018;18(1):49. doi:10.1186/s12883-018-1046-z

198. Latronico N, Guarneri B. Critical illness myopathy and neuropathy. *Minerva Anestesiol*. 2008;74(6):319-323.

199. Jolley SE, Bunnell AE, Hough CL. ICU-Acquired Weakness. *Chest*. 2016;150(5):1129-1140. doi:10.1016/j.chest.2016.03.045

200. Muhle P, Suntrup-Krueger S, Bittner S, et al. Increase of Substance P Concentration in Saliva after Pharyngeal Electrical Stimulation in Severely Dysphagic Stroke Patients - an Indicator of Decannulation Success? *Neurosignals*. 2017;25(1):74-87. doi:10.1159/000482002

201. Molina CA, Barreto AD, Tsivgoulis G, et al. Transcranial ultrasound in clinical sonothrombolysis (TUCSON) trial. *Ann Neurol*. 2009;66(1):28-38. doi:10.1002/ana.21723

202. Molina CA, Ribo M, Rubiera M, et al. Microbubble administration accelerates clot lysis during continuous 2-MHz ultrasound monitoring in stroke patients treated with intravenous tissue plasminogen activator. *Stroke*. 2006;37(2):425-429. doi:10.1161/01.STR.0000199064.94588.39

203. Nacu A, Kvistad CE, Naess H, et al. NOR-SASS (Norwegian Sonothrombolysis in Acute Stroke Study): Randomized Controlled Contrast-Enhanced Sonothrombolysis in an Unselected Acute Ischemic Stroke Population. *Stroke*. 2017;48(2):335-341. doi:10.1161/STROKEAHA.116.014644

204. Alexandrov AV, Köhrmann M, Soinne L, et al. Safety and efficacy of sonothrombolysis for acute ischaemic stroke: a multicentre, double-blind, phase 3, randomised controlled trial. *Lancet Neurol*. 2019;18(4):338-347. doi:10.1016/S1474-4422(19)30026-2

205. Alexandrov AV, Tsivgoulis G, Köhrmann M, et al. Endovascular equipoise shift in a phase III randomized clinical trial of sonothrombolysis for acute ischemic stroke. *Ther Adv Neurol Disord*. 2019;12:1756286419860652. doi:10.1177/1756286419860652

206. Juenemann M, Braun T, Schleicher N, et al. Neuroprotective mechanisms of erythropoietin in a rat stroke model. *Transl Neurosci*. 2020;11(1):48-59. doi:10.1515/tnsci-2020-0008

207. Dziewas R, Glahn J, Helfer C, et al. FEES für neurogene Dysphagien : Ausbildungscurriculum der Deutschen Gesellschaft für Neurologie und Deutschen Schlaganfall-Gesellschaft. *Nervenarzt*. 2014;85(8):1006-1015. doi:10.1007/s00115-014-4114-7

208. Dziewas R, Stellato R, van der Tweel I, et al. Pharyngeal electrical stimulation for early decannulation in tracheotomised patients with neurogenic dysphagia after stroke (PHAST-TRAC): a prospective, single-blinded, randomised trial. *The Lancet Neurology*. 2018;17(10):849-859. doi:10.1016/S1474-4422(18)30255-2

209. Braun T, Pukropski J, Yeniguen M, et al. Inter- and intra-rater reliability of computer-assisted planimetry in experimental stroke research. *J Neurosci Methods*. 2019;312:12-15. doi:10.1016/j.jneumeth.2018.11.012

210. Hamzic S, Braun T, Juenemann M, et al. Validation of the German Version of Functional Oral Intake Scale (FOIS-G) for Flexible Endoscopic Evaluation of Swallowing (FEES). *Dysphagia*. 2021;36(1):130-139. doi:10.1007/s00455-020-10114-1

211. Braun T, Juenemann M, Viard M, et al. Adjustment of oral diet based on flexible endoscopic evaluation of swallowing (FEES) in acute stroke patients: a cross-sectional hospital-based registry study. *BMC Neurol*. 2019;19(1):282. doi:10.1186/s12883-019-1499-8

212. Braun T, Juenemann M, Viard M, et al. Flexible endoscopic evaluation of swallowing (FEES) to determine neurological intensive care patients' oral diet. *Int J Speech Lang Pathol*. 2020:1-9. doi:10.1080/17549507.2020.1744727

8. Danksagung

Ich danke Herrn Prof. Dr. Manfred Kaps für die klinische und wissenschaftliche Ausbildung der letzten Jahre sowie Herrn Prof. Dr. Hagen Huttner für sein Interesse und die Unterstützung dieser Arbeit.

Herrn Prof. Dr. Tibo Gerriets und Frau PD Dr. Marlene Tschernatsch danke ich für die kreative und freundschaftliche Zusammenarbeit. Bei Herrn Prof. Dr. Christian Tanislav möchte ich ich für die anregenden Forschungsideen und die Einführung in die statistische Arbeit bedanken.

Ich danke meinen Freunden M.A. Samra Hamzic, Dr. Martin Jünemann, Dr. Maxime Viard, Frau Susanne Weber und Dr. Mesut Yenigün für die langjährige freundschaftliche Zusammenarbeit, Unterstützung und das Ertragen meines Wehklagens.

Herrn Dr. Mario Prosiegel möchte ich danken für den Gedankenaustausch und die vielen Ideen.

Außerdem möchte ich meinen tierärztlichen Kolleginnen Maaike Terhaar und Laura Sünner für die Hilfe im Rahmen der tierexperimentellen Arbeiten danken. Frau Johanna Wagner-Doerr danke ich für den wissenschaftlichen Austausch und die statistische Expertise danken.

Herrn Dieter Braun danke ich für die orthographische Korrektur der Arbeit.

Nicht zuletzt danke ich meiner Frau Jasmin, die mich in all den Jahren unterstützt und in der Zeit, die ich mit dieser Arbeit verbrachte, stets Rücksicht hatte. Hierfür nochmal ein besonderes Danke.

9. Erklärung

Hiermit erkläre ich, dass ich die mir zuzuordnenden Teile im Rahmen der kumulativen Habilitationsschrift selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nichtveröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Ich versichere, dass ich für die nach §2 (3) der Habilitationsordnung angeführten bereits veröffentlichten Originalarbeiten als Erst- oder Seniorautor fungiere, da ich den größten Teil der Daten selbst erhoben habe, für das Design der Arbeit verantwortlich bin und die Manuskripte maßgeblich gestaltet habe. Für alle von mir erwähnten Untersuchungen habe ich die in der „Satzung der Justus-Liebig-Universität zur Sicherung guter wissenschaftlicher Praxis“ niedergelegten Grundsätze befolgt. Ich versichere, dass alle an der Finanzierung der Arbeit beteiligten Geldgeber in den jeweiligen Publikationen genannt worden sind. Ich versichere außerdem, dass die vorliegende Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Weise einer anderen Prüfungsbehörde vorgelegt wurde oder Gegenstand eines anderen Prüfungsverfahrens war. Mit der Überprüfung meiner Arbeit durch eine Plagiatserkennungssoftware bzw. ein internetbasiertes Softwareprogramm erkläre ich mich einverstanden.

Gießen, im Mai 2021

Tobias Braun