The occurrence and prevention of CSF leakage

Application of a patch in different neurosurgical indications

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ISBN: 978-94-6483-231-0
Provided by thesis specialist Ridderprint, ridderprint.nl
Printing: Ridderprint
Layout and design: Harma Makken, persoonlijkproefschrift.nl

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Application of a patch in different neurosurgical indications

De incidentie en preventie van liquorlekkage

Het toepassen van een pleister bij verschillende neurochirurgische indicaties
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof.dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

dinsdag 17 oktober 2023 des middags te 4.15 uur

door

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geboren op 19 november 1992
te Amsterdam

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Dit proefschrift werd (mede) mogelijk gemaakt met financiële steun van Polyganics B.V.

Voor mijn ouders

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Introduction

Introduction

Cerebrospinal fluid (CSF) is the liquid surrounding the brain and spinal cord. The cerebrospinal fluid system is composed of the cerebral ventricles, the sulci and cisterns, and the cerebral and spinal subarachnoid space.[1] The clear and colorless fluid is contained within the meninges that form the border surrounding the central nervous system. CSF has several important functions. It is a shock absorber protecting the brain by proving buoyancy within the skull.[2] Furthermore, it provides nutrients, clears waste products of metabolism and plays a role in immunity.[2]

The total volume of CSF present in adults is approximately 150 mL.[1] CSF contains ions (Na^+, Cl^-, HCO_3^-, K^+, Ca^{++}, Mg^{++} and Mn^{++}), vitamins (Vitamin C, folate), peptides and proteins.[2] Under physiological conditions there are less than 5 white blood cells per mm^3 in CSF, a protein level of 20-40 mg/100 mL and glucose level of 60-80%.[3]

CSF is produced with a rate of 0.3-0.4 mL/min.[1] It is widely accepted that the majority of CSF is produced by the choroid plexus in the lateral ventricles and absorbed by the arachnoid villi. This classic hypothesis based on the joint research efforts of the founding fathers of neurosurgery, Weed, Dandy and Cushing, assumes a unilateral flow of CSF from the lateral ventricles, through the foramina of Monro, the third ventricle and aqueduct into the craniospinal subarachnoid space.[1–3] However, this hypothesis has been challenged by more recent publications.[4] It has now become understood that the motion of CSF is driven by systole-diastole pulsation, gravity and body posture and is bidirectional (oscillatory) rather.[1,4]

More controversy, however, continues to exist about the production and absorption of CSF. The discovery of the aquaporin which allows water to move freely into and out of the CSF has led to a new theory of CSF production and absorption[4]. In the Bulat-Klarica-Oreskovic hypothesis CSF production and absorption occur throughout the craniospinal axis through osmolarity gradients.[4]

When the natural barrier of the meninges is breached CSF may leak from the nose (rhinoliquorrhea), ear (otoliquorrhea) or surgical incision. Although CSF leakage may result from trauma (i.e. skull fracture) or occur spontaneously this thesis will consider CSF leakage as a complication of neurosurgical intervention only. CSF leakage is a serious complication after neurosurgical procedures, which may lead to poor wound healing, infection, CSF hypotension syndrome and pneumocephalus.[5–7] The definition of CSF leakage varies across the literature. It may include a subcutaneous CSF collection, also called pseudomeningocele, without a fistula or percutaneous leakage only. Although a pseudomeningocele may have psychological and physical

consequences of its own, it is often self-limiting. We will therefore consider it a separate entity. CSF leakage will be defined as the leakage of CSF through the skin incision.

CSF leakage after neurosurgery can be treated conservatively, with a pressure bandage and or placement of additional sutures across the incision at bedside. Invasive treatment strategies include reoperation for reclosure of the surgical wound or CSF diversion procedures. CSF diversion procedures aim to reduce the flow of CSF through the fistula by reducing the intracranial pressure and proving a drainage route for CSF with less resistance compared to the fistula. CSF diversion can be accomplished by placement of a temporary drain from the ventricular system to a pressure-controlled reservoir outside the body or placing such drain in the subarachnoid space at the level of the lumbar vertebrae. Permanent CSF diversion, by placing a shunt from the ventricular system or spinal subarachnoid space to the peritoneal cavity, may be desired in case of CSF leak in combination with hydrocephalus. The main drawback of any CSF diversion treatment is the risk of central nervous system infections.[8,9] Placement of an external CSF drain furthermore severely negatively influences patient mobility. Strict bedrest is often prescribed in these cases. Moreover, placement of an external ventricular drain (EVD) or permanent CSF diversion shunt requires general anesthesia and poses health risks such as intra ventricular hemorrhage and causes pain and discomfort. The placement of an external lumbar drain (ELD) mostly occurs under local anesthesia and poses the risk of a spinal epidural hematoma.

The incidence of CSF leakage after intradural cranial surgery in adults is reported to be 7.1% in a recent international multicenter historical cohort study.[10] Smoking, infratentorial surgery and the use of a dural substitute were identified as risk factors in this retrospective analysis.[10] Furthermore, younger age, male sex and higher body mass index (BMI) showed a significant association with CSF leakage, however, as the odds ratios (ORs) for these factors were close to 1, these were not considered clinically relevant.[10] The use of a dural sealant was associated with lower CSF leakage risk. Patients with CSF leakage had higher odds of wound infection and/or meningitis as compared to patients without. Moreover, treatment of CSF leakage was invasive in the majority of cases. It required placement of external CSF drain in 80% of cases and revision surgery in 32%.[10]

Incidence for CSF leakage after intradural spinal surgery reported in the literature is 5-13%.[11–13] In addition, unintended durotomy, resulting in a 10% CSF leakage risk, occurs in 1-2% of extradural cases.[14–17] These studies indicate that CSF leakage is a frequent and clinically significant complication in neurosurgery, for cranial as well as spinal surgery.

Watertight closure with sutures has historically been the primary strategy to prevent CSF leakage. However, even after meticulous suturing, leakage can occur between

sutures or through the needle holes. In recent years various products to augment dural closure by creating a watertight seal have been developed. Dural sealants can be made of biopolymers or synthetic polymers.[18] Sealants based on proteins, usually animal or human derived fibrinogen and thrombin, rely on clot formation with the underlying tissue to seal. There are some important drawbacks of using protein-based sealants; potential risk of viral or prion transmission, induction of an immunogenic reaction, the fact that they often require to be crosslinked with potentially neurotoxic components and their biodegradation is influenced by the site of implantation, availability and concentration of enzymes.[18] PEG-polymers are the most commonly used form of synthetic sealants. These consist of two components to form a liquid hydrogel which adheres to the underlying tissue. An important downside of PEG-polymers is that they may swell as a result of water uptake, potentially causing compression injury.[19–22] Yet, synthetic polymers offer the possibility of adjusting the features of the material to optimize the product. Ready-to-use patch sealants may be preferred over liquids in terms of user experience.[18]

The efficacy of dural sealants was evaluated in a meta-analysis comparing the CSF leakage rate between cranial cases with sealant use and without.[23] No significant differences in CSF leakage were observed between groups.[23] Yet, sealant use appeared to reduce the risk of surgical site infection. A similar study evaluating CSF leakage after spinal surgery also showed that there were no significant differences in CSF leakage between cases with and without dural sealants.[24] This result was found both for intended as well as incidental durotomy. Minimally invasive surgery, however, did have lower CSF leakage rates, independent of sealant use.[24]

The utility of nine commonly used dural sealants was compared and evaluated in an *in vitro* study.[25] To determine acute burst pressure an in vitro set up was modified from the ASTM F2392-04 by using porcine dura, artificial CSF, temperature matching physiological body conditions at 37 degrees Celsius and use of computer software to evaluate exact burst pressure. Resistance over time was evaluated using a 72-hours pressure pulse assay mimicking the standardized triphasic intracranial pressure waves.[25] In the acute burst pressure test only 3 sealants showed burst pressures above physiological intracranial pressure; Tachosil (Corza Health, San Diego, USA), Adherus (Stryker, Kalamazoo, USA) and Duraseal (Integra LifeSciences, Princeton, USA).[25] In the resistance test just two sealants maintained sufficient adherence to the dura over 72 hours; Duraseal and Adherus.[25]

Osbun et al. (2011)[26] compared the use of Duraseal, a PEG-hydrogel sealant, to standard of care in a randomized controlled trial. The control group consisted of different methods at the discretion of the surgeon; additional sutures, autologous dural grafts, off-label use

of various biological products including fibrin glue, gelatin and collagen sponges, dural substitutes, and hemostatic agents. The CSF leakage rate similar between both groups. There were no statistically significant differences in neurosurgical complications or surgical site infections. Duraseal application was faster compared to control.[26]

In another randomized controlled trial Duraseal was compared to Adherus, PEG-hydrogel sealant with similar application properties. The efficacy of both products was similar 90.6% vs. 91.2%, respectively.[27] Although expansion of the sealants was not evaluated with magnetic resonance imaging (MRI) in this trial, no adverse events (AEs) related to swelling of the sealant leading to neurological deficits were observed.[27] Preclinical testing of Adherus has shown limited volumetric expansion only.[28] Yet, for Duraseal compression injuries have been reported.[19,21,22] These involve posterior fossa and spine cases though.

Tachosil, a collagen-based sponge coated with human derived thrombin and fibrinogen, originally developed as a hemostatic agent, is commonly applied as a sealant in neurosurgery as well. Its efficacy in preventing CSF leakage compared to a control group consisting of sutures only was investigated in a randomized controlled trial. No statistically significant benefit was found for the addition of Tachosil to standard sutures in prevention of CSF leakage or surgical site infection.[29]

The results of the studies described above indicated that a critical attitude towards the use of the then available sealants was warranted given the additional costs involved and lack of evidence for their efficacy. At the same time, these studies demonstrated that there still exists an unmet clinical need for an effective and easy to use dural sealant.

A novel dural sealant, Dura Sealant Patch (DSP) (Liqoseal® (Polyganics B.V., Groningen)) has been developed to meet this need **(figure 1, chapter 3 page 44)**. The DSP is composed of a watertight biodegradable polyesterurethane (PU) layer and an adhesive layer of poly copolymer and multiarmed N-hydroxylsuccinimide (NHS) functionalized polyethylene glycol (PEG).[30, 31] The PEG-NHS adheres to the dura by interaction with the amine groups present. It is non-immunogenic and non-toxic.[30] Biodegradation has minimal site-to-site and patient-to-patient variation[30]. The expected biodegradation of the DSP is 1 year.[30, 31]

DSP was compared to the 3 commonly used FDA and/or CE approved sealants (Adherus, Duraseal and Tachosil) in a cranial and spinal *in vitro* set up, as described above.[30] The acute burst pressure of the DSP in the cranial model was higher than that of these clincally available sealants. In the spinal model burst pressure was higher than that of TachoSil, but not Adherus and Duraseal. Three-day resistance tests showed that 2 out of 3 the DSP in both models remained attached. This in vitro study shows that the DSP can form and maintain a watertight seal over a dural defect.[30]

Subsequentially, safety and biodegradability of the DSP as compared to other sealants was evaluated in an in vivo cranial porcine model.[31] DSP was implanted in 15 pigs. The comparison groups were composed of 11 pigs with no sealant and 6 pigs that received either Duraseal or Tachosil. Each group was subdivided into subgroups with survival times between 3 days and 12 months. Histological, MRI and clinical data were evaluated. The study concluded that the DSP did not swell with a maximum mean thickness of 2.1 mm at one month.[31] No percutaneous CSF leakage was observed in any of the animals.[31] An epidural CSF collection could not be excluded in one animal implanted with Liqoseal.[31] The resorption time of the DSP was between 6 months and 12 months postoperatively. Foreign body reaction induced by the sealants was similar across groups, yet more prolonged for DSP because of its slower degradation.[31] An advantage of the prolonged degradation, however, may be that it allows for ample time for the dura to heal whilst maintaining a watertight seal.[31] Based on the result of this animal study the DSP was deemed safe for intracranial use in a first-in-human study[31].

The first in-human study to evaluate the safety and efficacy of DSP in the prevention of CSF leakage after cranial surgery in adults (ENCASE) was conducted as an open-label single arm multicenter trial.[32,33] A total of 40 patients received DSP in addition to standard sutures as a means of dural closure and were followed-up for a total of 12 months. The primary composite endpoint was comprised of the following: postoperative percutaneous CSF leakage, intraoperative leakage at 20 cm H_2O positive end-expiratory pressure or postoperative wound infection. No patient met de primary endpoint.[33] MRI evaluation showed no clinically significant swelling of the device.[33] Based on these results DSP has been CE certified since January 2020.[33] The most important limitation of ENCASE was its single-arm design.[33] Therefore, a randomized trial is necessary to establish efficacy compared to current best practice.

As the use of a sealant requires financial input, it is important to assess the health economic consequences of CSF leakage and the potential impact of preventative strategies to assist decision making regarding their use. Moreover, DSP has thus far only been approved for cranial use in adults. Yet, CSF leakage is challenging complication in other surgical approaches as well. The anatomical and surgical characteristics of these specific approaches require separate evaluation of the potential use of DSP. Finally, children comprise a specific patient category, yet the incidence of CSF leakage and risk factors in pediatric patients has not been thoroughly investigated. This is an important first step to be able the determine the clinical need and specific requirements for application of the DSP in this patient category.

This thesis will consider the prevention CSF leakage using a novel dural sealant, Dura Sealant Patch, and evaluate its potential further applications. The following questions

will be addressed: 1. Is prevention of CSF leakage using strategies that require financial input beneficial from a health-economic point of view? 2. Is the DSP non-inferior compared to current best practice in preventing CSF leakage after cranial surgery? 3. Can the DSP be used for the prevention of CSF leakage in spinal surgery? 4. Can the DSP be used for the prevention of CSF leakage in transsphenoidal surgery? 5. What is the incidence of CSF leakage in the pediatric population and should the DSP be further developed for pediatric use? The latter research question is posed to provide insight in whether the questions raised above would also be applicable to children and what specific adjustments might be required.

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I

Safety and efficacy of Dura Sealant Patch in cranial surgery


2

Cerebrospinal fluid leakage costs after craniotomy and health economic assessment of incidence reduction from a hospital perspective in the Netherlands

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British Medical Journal Open. 2021. 11(12):e052553.
doi: 10.1136/bmjopen-2021-052553.

Abstract

Objectives

We aim to quantify the cost difference between patients with incisional cerebrospinal fluid (iCSF) leakage and those without after intradural cranial surgery. Secondly, the potential cost savings per patient when a decrease in iCSF leakage rate would be achieved with and without added costs for preventative measures of various price and efficacy are modelled.

Design

Health economic assessment from a hospital perspective based on a retrospective cohort study

Setting

Dutch tertiary referral center

Participants

We included 616 consecutive patients who underwent intradural cranial surgery between September 1st, 2017 and September 1st, 2018. Patients undergoing burr-hole surgery or transsphenoidal surgery, or who died within one month after surgery or were lost to follow up were excluded.

Primary and secondary outcome measures

Outcomes of the cost analysis include a detailed breakdown of mean costs per patient for patients with postoperative iCSF leakage and patients without, and the mean cost difference. For the scenario analyses the outcomes are the potential cost savings per 1,000 patients when a decrease in iCSF leakage would be achieved.

Results

Mean cost difference between patients with and without iCSF leakage was €9,665 (95%-Confidence Interval (CI), €5,125 to €14,205). Main cost driver was hospital stay with a difference of 8.5 days. A 25% incidence reduction would result in a mean cost saving of -€94,039 (95% CI -€218,258 to -€7,077) per 1,000 patients. A maximum cost reduction of -€653,025 (95% CI -€ 1,204,243 to -€169,120) per 1,000 patients could be achieved if iCSF leakage would be reduced with 75% in all patients, with 72 cases of iCSF leakage avoided.

Conclusions

Postoperative iCSF leakage after intradural cranial surgery increases healthcare costs significantly and substantially. From a health economic perspective preventative measures to avoid iCSF leakage should be pursued.

Key words

Neurosurgery, Health Economics

Strengths and limitations of this study

- To our knowledge this is the largest cost analysis providing a detailed breakdown of costs for iCSF leakage after intradural cranial surgery.
- An advantage of the method applied in this study is the adaptability of the transparent model to other settings.
- One limitation of our approach is the effect of initial surgery costs on the results of our analyses.
- Although, this analysis contains the largest patient population in an economic evaluation of iCSF leakage, the number of patients in the individual categories for secondary complications and treatment modalities remains low.

Introduction

Cerebrospinal fluid (CSF) leakage is one of the most common complications after neurosurgical intervention. The incidence of CSF leakage after intradural cranial surgery reported in the literature is 8% on average and depends on location of the surgery, indication of the surgery and patient-related risk factors[1] CSF leakage related complications include wound infection and meningitis, and may necessitate prolonged hospital admission, external CSF drainage or reoperation. Therefore, CSF leakage is not only associated with substantial morbidity, but with increased healthcare costs as well[1]. Grotenhuis found that the total extra cost of CSF leakage is approximately €12,000 for intradural cranial surgery, looking at the direct medical costs[2]. Previous research, however, lacks specification of the main cost drivers and analysis of costs for specific treatment modalities for CSF leakage. Both the health and economic consequences of CSF leakage emphasize the importance of prevention of CSF leakage.

Yet, preventative measures to reduce CSF leakage incidence may require financial input as well. Neurosurgeons closing themselves instead of residents, the use of devices, or increased operating room time because of a more precise closing technique to prevent CSF leakage may all lead to increased health care costs. Cost-benefit analyses of preventative strategies to reduce CSF leakage are lacking in the current body of literature.

In an increasingly cost aware health care system financial implications of complications and their prevention are of great importance in deciding which preventative strategies to pursue. Therefore, the health economic consequences should be considered as well when evaluating the efficacy of preventative strategies to avoid iCSF leakage.

The primary objective of the current study is to quantify the difference in health care consumption and associated costs between patients with CSF leakage after intradural cranial surgery and those without postoperative CSF leakage. The secondary objective is to quantify the economic effect per patient when a decrease in CSF leakage rate and related complications would be achieved using preventative measures that may require financial input.

Methods

This cost analysis was performed from a hospital perspective, including detailed health care consumption of every individual patient. This study uses direct medical costs, without taking into account health insurance reimbursement.

Clinical data from a single center were retrieved from previously collected retrospective international multicenter database (unpublished raw data). All consecutive adult

patients undergoing intradural cranial surgery between 1 September 2017 and 1 September 2018 at the University Medical Center Utrecht were included. Patients who died within one month after surgery or were lost to follow up were excluded, as for these patients there was insufficient certainty regarding the occurrence of the primary outcome measure (CSF leakage) introducing bias into the analysis and health care resources utilized during follow-up. Patients undergoing burr-hole surgery or transsphenoidal surgery were excluded, as they represent separate patient categories with specific health care utilization.

The following surgical characteristics had been collected: indication, urgency level, reoperation (yes/no), location of craniotomy (supra- or infratentorial), use of dural substitute and use of a dural sealant. Patient characteristics retrieved from the database included: age, sex, pre-operative dexamethasone use, history of radiation therapy, diabetes, BMI and smoking.

CSF leakage was defined as incisional cerebrospinal fluid (iCSF) leakage (either clinically diagnosed or confirmed through Beta-2 transferrin test) and did not include pseudomeningocele. Postoperative infection included superficial wound infection and deep wound infection and/or meningitis requiring treatment. The type of treatment was reviewed when iCSF leakage occurred. The treatment was divided into three categories: conservative treatment, external drainage placement and operative wound revision. Conservative treatment consisted of pressure bandage for wound compression and/or additional suture placement. Firstly, a cost analysis was performed based on clinical and detailed cost data. This cost analysis was followed by scenario analyses to investigate the effect of reduction of iCSF leakage on health economic outcomes. A decision tree was used to combine the aforementioned cost analysis and the incidence rates of complications.

Cost Analysis

Healthcare resources consumed by eligible patients from 30-days prior to 180-days after surgery were retrieved from medical records. Costs included readmissions and considers all-cause healthcare utilization. Unit prices were retrieved from the Dutch Healthcare Authority (Nederlandse Zorgautoriteit; NZA), the cost-manual of the National Healthcare Institute (Zorginstituut Nederland; ZiN) and literature research and linked to the corresponding healthcare activities[3,4]. The costs for an external ventricle drain and external lumbar drain and dural sealants were based on existing literature and local prices[5]. Costs for cranial surgery and reoperation were determined based on operating room time multiplied by cost per minute (€10,59)[6]. All costs are presented in 2018 Euros.

Outcomes of the cost analysis included a detailed breakdown of mean costs per patient for patients with postoperative iCSF leakage and patients without. Different costs were

divided into categories; outpatient visits, diagnostics, primary surgery, expensive drugs (e.g., chemotherapy for brain tumor patients), clinical admissions, other costs (e.g physiotherapy and dietetics), leakage treatment and sealant costs.

As well as the total healthcare costs for patients with CSF leakage stratified by treatment; reoperation, drain (external lumbar drain and external ventricle drain), reoperation and drain, and/or conservative treatment (including pressure bandage and additional sutures). Difference between groups was tested with Mann-Whitney-U since data was not normally distributed.

Scenario Analysis

Model Development

A decision tree was developed **(Supplementary Material 1)** outlining intradural cranial surgery and the occurrence of complications, including iCSF leakage. This decision tree allows the quantification of the room for improvement in scenario analyses by adapting probabilities of individual events. This is achieved by multiplying the probability of a patient qualifying for a certain subgroup by the healthcare costs associated with these subgroups. **Supplementary Material 1** outlines the probabilities and subgroup costs used to recalculate healthcare costs. Outliers can impact outcomes significantly. To account for input parameter uncertainty distributions were fitted, beta distributions for probabilities and gamma distributions for costs. A probabilistic analysis with a Monte Carlo simulation with 10,000 iterations was used to determine model outcomes and ranges.

Scenario Analyses

Scenario analyses were performed to determine the health economic effects of reduction of iCSF leakage. Three different scenarios were applied to gain more information on the possible benefits of CSF reduction with various preventative strategies. (I) The iCSF leakage incidence use was decreased with 25% steps between 0% and 75%. (II) The iCSF leakage incidence was reduced and weighted against varying costs of potential interventions of variable efficacy. (III) The first two scenario's applied for subgroups with different risk of iCSF leakage (supratentorial surgery and infratentorial surgery). Outcomes of the scenario analyses were presented as difference in costs and number of iCSF leakage cases avoided per 1,000 patients was calculated as well as the number needed to treat (NNT). To determine parameter influence on the outcome of the scenarios, a deterministic sensitivity analysis was performed and a tornado diagram was constructed.

Patient and Public Involvement

No patients involved.

Results

In total 616 consecutive patients were included in this study. **Table 1** provides an overview of the patient characteristics. Mean age of patients was 53.5 (±15.8) years. The most common indication for surgery was tumor resection; 399 patients (64.8%) and most patients had a supratentorial approach; 517 (83.9%). A total of 59 patients had postoperative iCSF leakage (9.6%).

Supplementary Material 1 Input parameters for the model of the scenario analysis

Table 1. Patient characteristics

	All patients (N=616)	No iCSF leakage (N=557)	iCSF leakage (N=59)	P-value
Male; ***N (%)***	296 (48.1)	267 (49.7)	29 (49.2)	0.859
Age; ***Years (±SD)***	53.5 (±15.8)	53.6 (± 15.8)	52.6 (± 16.2)	0.656
BMI; ***(±SD)***	26.1 (± 6.9)	25.9 (± 6.9)	27.8 (± 6.4)	0.036
Indication; ***N (%)***				0.474
Tumor	399 (64.8)	356 (63.9)	43 (72.9)	
Vascular	121 (19.6)	113 (20.3)	8 (13.6)	
Epilepsy	62 (10.1)	57 (10.2)	5 (8.5)	
Trauma	22 (3.6)	19 (3.4)	3 (5.1)	
Other	12 (1.9)	12 (2.2)	0 (0.0)	
Tentorial approach; ***N (%)***				<.001
Supratentorial	517 (83.9)	481 (86.4)	36 (61.0)	
Infratentorial	99 (16.1)	76 (13.6)	23 (39.0)	

BMI: body mass index
iCSF: incisional cerebrospinal fluid
SD: standard deviation
P-Values smaller than 0.05 are considered significant

Cost per patient and detailed breakdown costs

Average cost per patient and a detailed breakdown of costs are included for all of the 616 patients. In **table 2**, the average costs per patient with and without iCSF leakage are outlined. Five out of 7 cost categories were higher for patients with iCSF leakage compared to patients without iCSF leakage. Costs for external ventricle drain, external lumbar drain and reoperation were categorized under treatment costs in **table 2**.

Table 2. Healthcare costs for patients with and without iCSF leakage and the difference (N=616)

	No iCSF leakage (N=557)		iCSF leakage (N=59)		Difference	P-value
	Mean	(95%-CI)	Mean	(95%-CI)		
Primary surgery	€ 1,958	(€ 1,882 to € 2,035)	€ 2,439	(€ 2,102 to € 2,776)	€ 481	0.007
Out patient visits	€ 1,696	(€ 1,570 to € 1,821)	€ 2,006	(€ 1,631 to € 2,380)	€ 310	0.132
Diagnostics	€ 2,360	(€ 2,215 to € 2,505)	€ 2,903	(€ 2,214 to € 3,592)	€ 543	0.032
Expensive drugs	€ 948	(€ 572 to € 1,324)	€ 812	(€ 135 to € 1,489)	-€ 136	0.821
Clinical admissions	€ 10,701	(€ 9,806 to € 11,597)	€ 17,568	(€ 12,642 to € 22,494)	€ 6,867	0.004
Others	€ 2,703	(€ 2,377 to € 3,030)	€ 3,844	(€ 2,638 to € 5,050)	€ 1,141	0.06
Leakage treatment	€ 0	(€ 0 to €0)	€ 474	(€ 354 to € 595)	€ 474	<.001
Sealant	€ 131	(€ 116 to € 146)	€ 117	(€ 72 to € 161)	-€ 14	0.555
Total	€ 20,498	(€ 19,183 to € 21,813)	€ 30,163	(€ 23,654 to € 36,672)	€ 9,665	0.005

iCSF: incisional cerebrospinal fluid
Others includes physiotherapy and dietetics.
P-Values smaller than 0.05 are considered significant

Difference in costs between patients without iCSF leakage and with iCSF leakage was €9,665 (95%-Confidence Interval (CI), €5,125 to €14,205). Total average healthcare costs for patients without iCSF leakage was €20,498 (95%- CI; €19,183 to €21,813) compared to €30,163 (95%-CI; €23,654 to €36,672) for patients with iCSF leakage **(table 2)**. When comparing costs incurred starting from the day of primary surgery (days 0-180), costs were €17,759 (95%- CI; €16,497 to €19,021) for patients without iCSF leakage and €28,105 (95%- CI; €21,695 to €34,515) for patients with iCSF leakage.

Main reason for the difference in cost, over both the total time and the post-operative time, was the significant difference in length of hospital stay, for which costs are categorized as clinical admissions. Difference in length of stay (LOS) was 8.5 days (95%-CI; 5.3 to 11.7). For patients without incisional leakage LOS was 12.8 (95%-CI: 11.9 to 13.8) days and for patients with iCSF leakage LOS was 21.3 (95%-CI: 16.6 to 26.1) days. Furthermore, the incidence of secondary complications was significantly higher in the iCSF group. Highest costs among subgroups were found for patients with deep wound infection and/or meningitis (€39,323-€57,862). Patients without additional complications had lowest costs among all subgroups (€19,050- €26,797) **(table 3)**.

For supratentorial surgery there was a significant cost difference between patients with iCSF leakage (€20,180, ± €14,504) and those without (€31,219 ± €25,224). For infratentorial surgery patients with iCSF leakage had a mean cost of €28,510 (± €25,057) as compared to €22.512 (± €22,369) for patients without iCSF leakage. This difference was not statistically significant **(table 3)**.

Table 3. Average total healthcare costs per patient for different subgroups based on approach and complication

	No iCSF leakage (N=557)			iCSF leakage (N=59)			P-value
	N	Mean	SD	N	Mean	SD	
Supratentorial	481	€ 20,180	€ 14,504	36	€ 31,219	€ 25,224	0.014
No complications	457	€ 19,050	€ 12,844	18	€ 26,797	€ 19,547	0.015
Superficial wound infection	10	€ 31,616	€ 32,380	10	€ 30,448	€ 22,020	0.926
Deep wound infection and/or meningitis	14	€ 48,881	€ 24,918	8	€ 42,130	€ 37,982	0.557
Infratentorial	76	€ 22,512	€ 22,369	23	€ 28,510	€ 25,057	0.276
No complications	73	€ 21,883	€ 22,383	16	€ 25,163	€ 16,561	0.574
Superficial wound infection	2	€ 27,804	€ 11,027	2	€ 28,248	€ 16,988	0.978
Deep wound infection and/or meningitis	1	€ 57,862	-	5	€ 39,323	€ 50,566	0.755

iCSF: incisional cerebrospinal fluid
SD: standard deviation
P-Values smaller than 0.05 are considered significant

In the group of patients with postoperative iCSF leakage (N=59), 18 patients received conservative treatment, 7 patients required reoperation, 26 patients were treated with an external CSF drain and 8 patients required reoperation and a drain. In the group of patients treated conservatively 10/18 had CSF leakage once or twice. All other patients with CSF leakage had continuous leakage. **Table 4** shows the total healthcare costs and LOS for patients with iCSF leakage stratified per treatment modality. Lowest costs were found for the 18 patients who were treated conservatively (€21,046 (± €11,433)). Highest costs were found for the 7 patients requiring reoperation; €36,117 (± €45,056). Longest LOS was for patients requiring reoperation and drain; 26.5 days (± 17.6 days). There was no statistically significant difference in LOS or costs between patients who were treated conservatively and those who underwent reoperation. Patients who were treated with external CSF drainage or reoperation and external CSF drainage combined had significantly longer LOS and higher costs compared to patients who were treated conservatively. No significant differences in LOS or costs were found between invasive treatment modalities.

Table 4. Total healthcare costs and LOS for patients with iCSF leakage stratified by treatment

	Conservative treatment (N=18)	Surgery (N=7)	Surgery + drain (N=8)	Drain (N=26)	All patients (N=59)	Conservative vs. Surgery	Conservative vs. Surgery + drain	Conservative vs. Drain	Surgery vs. Surgery + drain	Surgery vs. Drain	Surgery + drain vs. Drain
						P-value					
Mean costs	€ 21,046	€ 36,117	€ 36,007	€ 33,073	€ 30,163	0.976	0.027	<.001	0.463	0.450	0.327
Costs SD	€ 11,433	€ 45,056	€ 21,490	€ 25,544	€ 24,977						
Mean LOS (days)	11.3	26.1	26.5	22.1	19.9	0.495	0.015	<.001	0.232	0.531	0.270
LOS SD	6.8	36.7	17.6	16.1	18.4						

iCSF: incisional cerebrospinal fluid
LOS: length of stay
SD: standard deviation
vs.: versus
P-Values smaller than 0.05 are considered significant

Scenario Analyses

Table 5 presents an overview of the outcomes of scenario analysis I and III.

Table 5. Results of the scenario analysis: difference in healthcare costs and cases avoided per 1,000 patients and number needed to treat to prevent one iCSF leakage case

Scenario	Incidence Change	Difference in Healthcare costs per 1,000 patients		% Runs Saving[a]	Cases of iCSF Leakage Avoided per 1,000		
		Mean	95% CI		Mean	95% CI	NNT
All Patients							
1	-25%	-€ 216,609	(-€ 402,445 to -€ 62,204)	99.71%	24	(18.45 to 30.00)	42
2	-50%	-€ 434,882	(-€ 821,229 to -€ 115,466)	99.61%	46	(37.16 to 59.91)	21
3	-75%	-€ 653,025	(-€ 1,204,243 to -€ 169,120)	99.73%	72	(54.97 to 89.89)	14
Supratentorial Only							
1	-25%	-€ 193,849	(-€ 371,531 to -€ 52,404)	99.83%	18	(12.58 to 23.23)	57
2	-50%	-€ 387,929	(-€ 746,597 to -€ 108,662)	99.83%	35	(25.00 to 46.33)	29
3	-75%	-€ 580,844	(-€ 1,112,175 to -€ 166,133)	99.87%	53	(37.44 to 69.81)	19
Infratentorial Only							
1	-25%	-€ 342,726	(-€ 1,095,834 to € 271,575)	85.38%	57	(37.58 to 79.55)	17
2	-50%	-€ 681,934	(-€ 2,203,623 to € 526,998)	84.81%	115	(76.19 to 160.71)	9
3	-75%	-€ 1,036,407	(-€ 3,276,620 to € 834,232)	85.24%	172	(114.64 to 240.03)	6

[a] Percentage of Monte Carlo simulations, percentage of runs out of 10,000, in which the scenario was cost saving compared to current standard care
CI: confidence interval
iCSF: incisional cerebrospinal fluid
NNT: number needed to treat

Figure 1 shows the potential cost savings per patient when a decrease in iCSF leakage would be achieved. A maximum cost reduction of -€653,025 (95% CI -€ 1,204,243 to -€169,120) per 1,000 patients could be achieved if iCSF leakage would be reduced with 75%. The number of cases avoided would be 72. The number needed to treat in this scenario is 14. For supratentorial surgery reduction of iCSF leakage with 25% to 75% would lead to significant cost reduction and a maximum of 53 cases of iCSF leakage avoided. For infratentorial surgery there is a trend towards substantial cost savings for reduction rates between 25-75%, however, this is not significant.

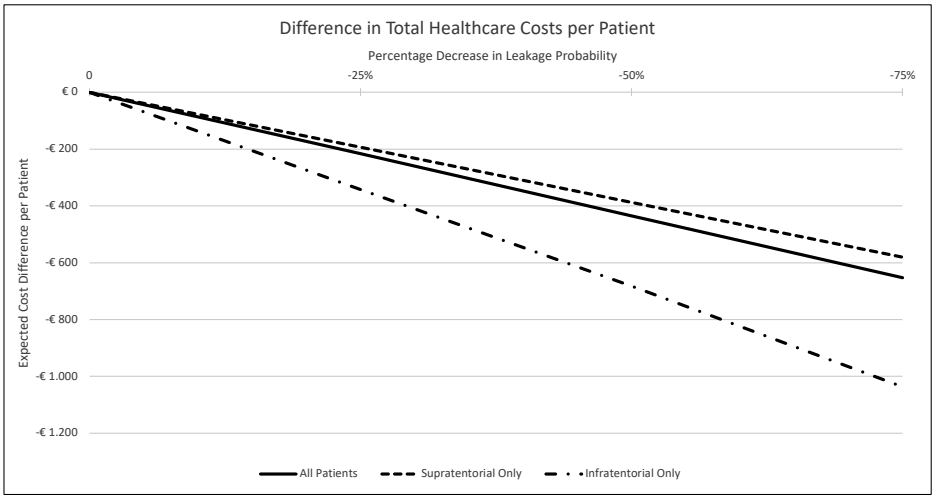

Figure 1. Potential costs savings per patient with a decrease in iCSF leakage

If costs of potential preventative strategies are added to accomplish iCSF leakage (scenario II and III) our model shows cost reduction for measures at a price of €250 per patient at an iCSF leakage reduction of 50-75% in all patients and both subgroups. Preventative strategies at a price of €500 euro per patient only lead to cost savings in all patients and supratentorial cases if they reduce iCSF leakage with 75%. For infratentorial cases this scenario results in cost savings at a 50% reduction as well. Preventative strategies that cost €750 per patient lead to cost reduction only when applied in infratentorial cases with an iCSF leakage reduction of 75% (**Figure 2**).

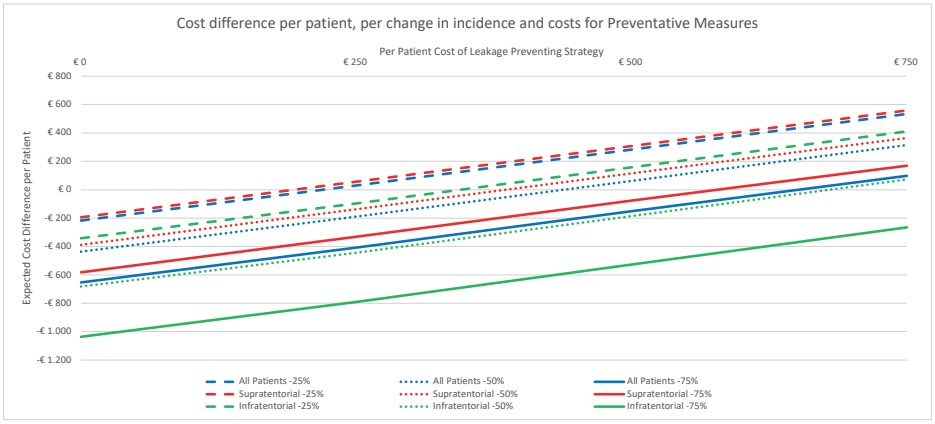

Figure 2. Cost difference per patient, per change in incidence and cost for preventative measure

The deterministic sensitivity analysis showed that the parameter with the greatest influence on scenario outcomes was costs for patients without iCSF leakage and an infratentorial approach. Lowest influence was found for the incidence of iCSF leakage in infratentorial patients (**Figure 3**).

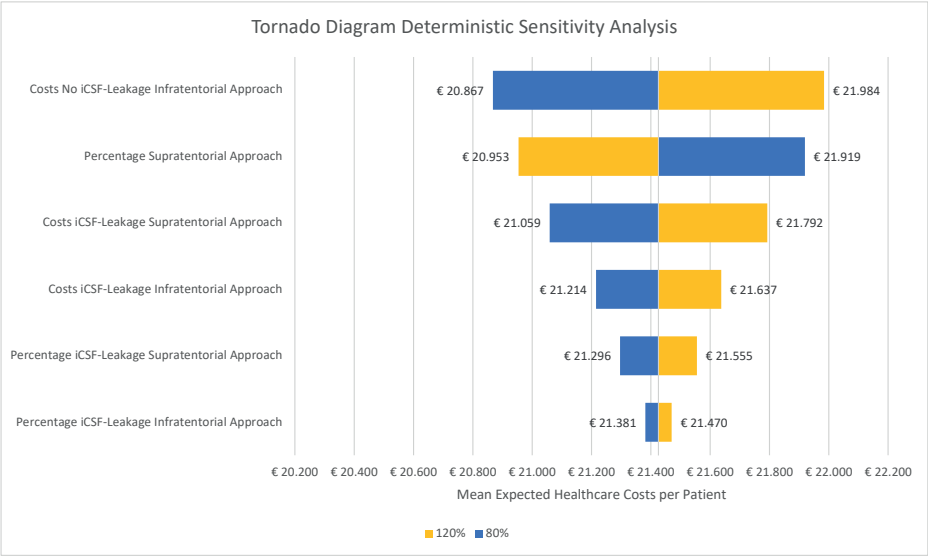

Figure 3. Tornado diagram indicting the influence of the different input parameters of the model

Discussion

There is a substantial and significant cost difference of €9,665 between patients with postoperative iCSF leakage after intradural cranial surgery and those without. The average healthcare cost for cranial intradural surgery ranges between €20,498 for patient without iCSF leakage and €36,117 for patients with reoperation, which was the most expensive. A maximum cost reduction of -€653,025 (95% CI -€ 1,204,243 to -€169,120) per 1,000 patients could be achieved if iCSF leakage would be reduced with 75% in all patients.

Our model shows that reducing leakage rates could lead to substantial cost reduction, even if financial input is required. However, whether the use of preventative measures that require financial input in all patients or a subgroup of patients a risk results in cost savings depends on their price and efficacy. Because of the higher risk of iCSF leakage in infratentorial surgery more expensive preventative measure of a certain efficacy could still lead to cost savings in this subgroup, when they are not for the total population.

To our knowledge this is the largest cost analysis providing a detailed breakdown of costs for iCSF leakage after intradural cranial surgery. Furthermore, it is the first study applying a model to calculate the health economic effects of improved preventative measures. An advantage of the method applied in this study is the adaptability of the transparent model to other settings. If other hospitals are aware of their leakage rate and healthcare costs, this method could be used to estimate possible future cost savings, for example with improved sealants.

One limitation of our approach is the effect of initial surgery costs on the results of our analyses. Despite this being the most comprehensive method of taking into account all associated costs, it may be the case that part of the cost difference is driven by the initial surgery, as complex and longer surgeries are more expensive. Secondly, we have collected health care consumption in a single center. There is thus a theoretical risk of missing the costs of patients that may have received follow-up treatment elsewhere, without this being communicated to the primary center. As patients with loss to follow-up were excluded from the initial database and treatment of complications in a different center is unusual, we do not believe this has affected the outcomes of the current study.

Thereby, although this analysis contains the largest patient population in an economic evaluation of iCSF leakage, the number of patients in the individual categories for secondary complications and treatment modalities remains low. It is therefore difficult to interpret cost differences for specific secondary complications in detail. In these limited numbers of cases heterogeneity of patients could be the main difference between those with iCSF leakage and those without. Results of the comparisons between the different treatment modalities should be interpreted with some caution as well, for the same reason. Especially, the subgroup of patients who underwent reoperation is limited in size and has large standard deviation of both the LOS and the costs. Furthermore, these limited subgroups led to larger uncertainty around the scenario analyses modeling the potential health economic effects of iCSF leakage reduction, especially for the infratentorial subgroup. Another limitation of the scenario analyses is the linear reduction in iCSF leakage, which assumes that iCSF leakage can be prevented with a certain efficacy across the total population. It may however be the case that for certain subgroups iCSF leakage cannot be avoided with preventative measures.

These results are based on healthcare consumption and costs of one center in the Netherlands. Therefore, applying these results to different countries is challenging. Differences in clinical practice and prices, for instance, may influence the effects observed in this study considerably[7]. It is thus recommended that data on cost prices and resource use should be obtained from or adapted to the setting of interest[7]. Furthermore, baseline risk should be location specific, whereas treatment effect may

be more generalizable[7]. Although, larger differences are to be expected between the healthcare systems across continents, even within western Europe economic analyses of medicines vary significantly[7].

The additional healthcare costs for patients with incisional CSF leakage in this study are comparable to those found by Grotenhuis (2005) in the Netherlands, who found a cost difference of approximately €12,000, for cranial surgery including transsphenoidal procedures[2]. Our study includes all healthcare resources consumed within a predefined time frame, whereas Grotenhuis based calculations on certain cost categories only. Another study from Germany by Piek et al. (2012) calculated cost differences between patients with and without CSF leakage in detail and found a comparable result of €11.420[8]. Their study, however, also included subcutaneous CSF collections as CSF leaks and it has a limited sample size of 168 patients (of which only three had percutaneous CSF leaks)[8].

The breakdown of costs shows that clinical admission is the main cost driver for the difference between patients with and without iCSF leakage. Patients with iCSF leakage have higher risk of infection or meningitis[9]. These complications may further explain the cost difference between patients with and without iCSF leakage as they require prolonged clinical admission. These results are in line with the study of Parikh et al. (2020) that identified increased LOS and the association of CSF leakage with secondary complications such as meningitis as the main reasons for increased healthcare costs after transsphenoidal surgery[10].

Additionally, the costs for interventional treatment of iCSF leakage are a substantial cost driver, considering that patients who can be managed conservatively have total average costs that are comparable to patients without iCSF leakage. In the group of patients managed conservatively, though, 10/18 patients (55.6%) did not have continuous iCSF leakage, but incisional leakage that occurred once or twice, suggestive of a subcutaneous pocket that has discharged. All patients that had to be managed with invasive treatment had continuous iCSF leakage. Patients treated with an external CSF drain have significantly longer LOS and higher costs compared to those treated conservatively. Contrary to Parikh et al. (2020) we did not find shorter LOS in patients treated with reoperation compared to those treated with external CSF drainage only[10]. This may imply that reoperation as a treatment for iCSF leakage is performed sooner after endoscopic endonasal surgery than after craniotomy. An advantage of reoperation compared to external CSF drainage is the quick return to mobilization as opposed to bedrest required during external CSF drainage. This is not reflected in a difference in LOS between these patients in our population, however. Besides a delay in surgical

treatment, other factors related to recovery such as comorbidity may explain why LOS is similar for these treatment modalities.

This study confirms that from a health economic perspective iCSF leakage should be reduced. Improved preventative strategies reducing the iCSF leakage rate, even though they may add to the overall healthcare costs per patient, could be beneficial from an economic standpoint. Furthermore, increased understanding of risk factors for iCSF leakage and associated costs may contribute to improving the indication for use of currently available and future methods of augmented dural closure. Considering that conservative treatment for continuous iCSF leakage is rarely effective, early interventional treatment for this group is recommended. Furthermore, methods that shorten LOS for patients with external CSF drains should be investigated. Our model of the health economic effects of iCSF leakage and potential cost savings of improved preventative strategies should be applied to different healthcare settings to evaluate the cost difference and potential cost savings location specifically to assist physicians and healthcare managers in decision making regarding preventative strategies to avoid iCSF leakage in their situation.

Declarations

Author Contributions

Conceptualization: TPCvD, GWJF; Methodology: GWJF, CvL, EMHS, TPCvD; Formal analysis and investigation: CvL, EMHS; Writing - original draft preparation: CvL, EMHS; Writing - review and editing: TPCvD, GWJF, EWH, AK, MK, CvL, EMHS; Resources: AK, MK; Supervision: TPCvD, GWJF, EWH.

Standards of reporting

The authors adhered to the CHEERS guidelines for economic reporting.

Funding

EMHS and AK received a research grant through Polyganics B.V., grant number N/A.

CvL and GWJF received a consultancy fee through Polyganics B.V. for this research.

Conflicts of interest

TPCvD is a consultant for Polyganics B.V..

Ethics statement

The retrospective chart review study involving human participants that the clinical data in this study have been retrieved from was performed in accordance with the institutional research committee. This study does not involve human participants.

Data sharing statement

The data set that was analysed for this study is available from the corresponding author upon reasonable request.

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Supplementary Material 1. Input parameters for the model of the scenario analysis & Model figure

Table 1. Probabilities used to recalculate healthcare costs.

	Probability			Costs		
	Mean	SE	Distribution	Mean	SE	Distribution
Supratentorial	0,839	0,015	Beta			
iCSF leakage	0,07	0,011	Beta	€ 31.218,73	€ 4.203,97	Gamma
No iCSF leakage	0,93	0,011	Beta	€ 20.179,66	€ 661,32	Gamma
Infratentorial	0,161	0,015	Beta			
iCSF leakage	0,23	0,043	Beta	€ 28.509,71	€ 5.224,84	Gamma
No iCSF leakage	0,77	0,043	Beta	€ 22.512,13	€ 2.565,91	Gamma

SE: standard error
iCSF: incisional cerebrospinal fluid

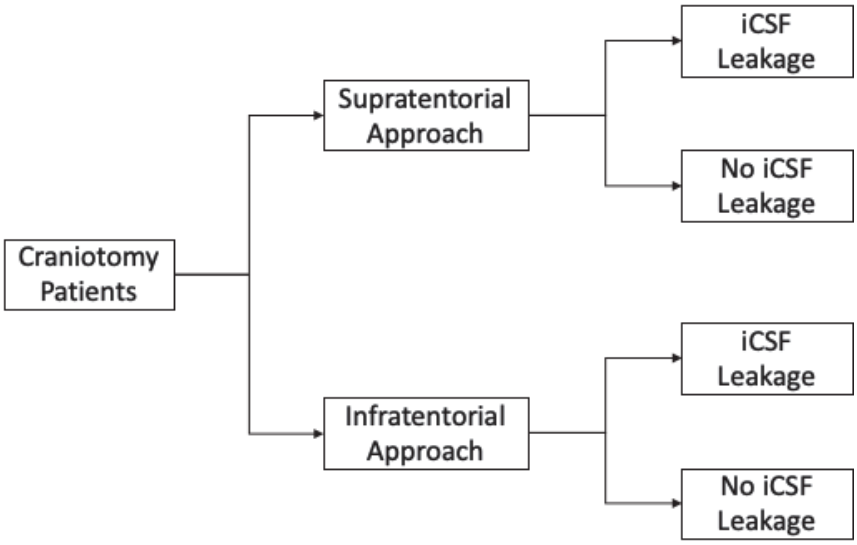

Figure 1. Graphic representation of model for scenario analyses.


3

Evaluate the safety and efficacy of Dura Sealant Patch in reducing cerebrospinal fluid leakage following elective cranial surgery (ENCASE II): study protocol for a randomized, two-arm, multicenter trial

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Trials. 2022; 23(1):581. https://doi.org/10.1186/s13063-022-06490-8

Abstract

Background

Cerebrospinal fluid (CSF) leakage is a frequent and challenging complication in neurosurgery, especially in the posterior fossa, with a prevalence of 8%. It is associated with substantial morbidity and increased healthcare costs. A novel dural sealant patch (Liqoseal) was developed for watertight dural closure. The objective of this study is to clinically assess the safety and effectiveness of Liqoseal as a means of reducing intra- as well as post-operative CSF leakage in patients undergoing elective posterior fossa intradural surgery with a dural closure procedure compared to the best currently available dural sealants.

Methods

We will conduct a two-arm, randomized controlled, multicenter study with a 90-day follow-up. A total of 228 patients will be enrolled in 19 sites, of which 114 will receive Liqoseal and 114 an FDA approved PEG sealant. The composite primary endpoint is defined as intraoperative CSF leakage at PEEP 20 cm H_2O, percutaneous CSF leakage within 90 days of, wound infection within 90 days of or pseudomeningocele of more than 20cc on MRI or requiring intervention. We hypothesize that the primary endpoint will not be reached by more than 10 patients (9%) in the investigational arm, which will demonstrate non-inferiority of Liqoseal compared to control.

Discussion

This trial will evaluate whether Liqoseal is non-inferior to control as a means of reducing CSF leakage and safety.

Trial registration

Clinicaltrials.gov, NCT04086550. Registered September 11, 2019

Keywords

CSF leakage, dura, sealing, prevention

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	Evaluate the Safety and Efficacy of Dura Sealant Patch in Reducing Cerebrospinal Fluid Leakage Following Elective Cranial Surgery (ENCASE II): study protocol for a randomized, two-arm, multicenter trial
Trial registration {2a and 2b}.	Clinicaltrials.gov, NCT04086550
Protocol version {3}	Protocol version 2.0, February 2021
Funding {4}	Polyganics BV Rozenburglaan 15A 9727 DL Groningen, The Netherlands
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Name and contact information for the trial sponsor {5b}	Polyganics BV Rozenburglaan 15A 9727 DL Groningen, The Netherlands
Role of sponsor {5c}	Sponsor co-designed the study with the authors. The sponsor, who is funding the study, is involved in site selection and day-to-day performance of the trial with regards to device accountability and study training. The regulatory submissions, data monitoring and data analysis is performed by a contract research organization (CRO). Interpretation of the data is performed in accordance with the coordinating investigators. The sponsor integrates the information provided by the CRO and coordinating investigators into the study report, which is reviewed by the CRO and coordinating investigators for final approval of the report. The authors have full freedom in writing and submitting the academic report. Draft material should be provided to the sponsor for review at least 30 days prior to submission or presentation date. The sponsor may require that the Investigators delete from their documents any reference to the sponsor's confidential information.

Introduction

Background and rationale {6a}

Cerebrospinal fluid (CSF) leakage is a frequent and challenging complication in neurosurgery, with a prevalence of 8%[1]. Risk factors include posterior fossa surgery, the size of the durotomy and patient-related factors such as immune-status[2]. It is associated with substantial morbidity and increased healthcare costs, estimated at $10,000-15,000 per patient per leakage[3]. CSF related complications include delayed wound healing, meningitis and surgical site infection which often require prolonged hospital stay, antibiotic treatment, reoperation or external lumbar drainage. To prevent CSF leakage, various dural sealants were developed to augment watertight closure of the dura. Thus far, their use has not shown a significant effect in reducing the number of complications[1].

The sponsor of this study has developed a dural sealant patch (Liqosael®, Polyganics BV) **(Figure 1)**. Liqoseal is designed to serve as an adjunct to primary dural closure in cranial surgery. Preclinical studies have shown that Liqoseal has advantages in dural adherence and burst pressure compared to other sealants[4]. The first in-human study (ENCASE) showed that Liqoseal is safe and easy to use[5]. However, a clinical comparative study testing its efficacy compared to control in humans has not been performed yet.

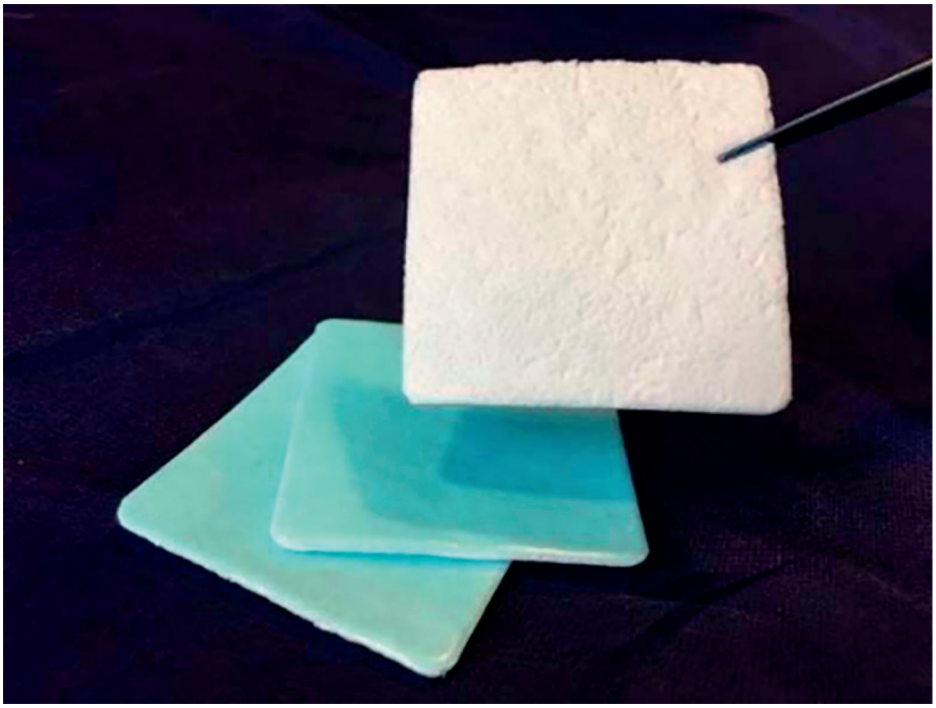

Figure 1. Liqoseal, investigational device. Length 8 cm, width 8 cm, and weight 1600 to 2000 mg.

Objectives {7}

The objective of the current ENCASE II study is to clinically assess the safety and effectiveness of Liqoseal as a means of reducing CSF leakage in patients undergoing elective posterior fossa intradural surgery, by showing non-inferiority compared to a control group.

Trial design {8}

This study protocol adheres to the SPIRIT reporting guidelines. The SPIRT checklist is supplemented as supplementary file 1. This study follows a randomized controlled, international, multicenter design with a 90-day follow-up. Patients will be randomized to receive Liqoseal or control (DuraSeal® (Integra) or Adherus® (HyperBranch, Medical Technology)) to be applied after the primary closure of the dura with suturing. Patients will be allocated in a 1:1 ratio to interventional device or control. The framework of this trial is noninferiority.

Methods: Participants, interventions and outcomes

Study setting {9}

This study will be conducted at up to 20 high volume neurosurgical centers in the United States of America and Europe. A complete list of participating sites can be found in appendix I.

Eligibility criteria {10}

Preoperative inclusion criteria for participants

- Patients who are able to provide written informed consent prior to participating in the clinical investigation.
- Age ≥ 22 years.
- Patients who are able to comply with study requirements.
- Patients scheduled for elective surgery including a trepanation to reach the subdural infratentorial space (with lower limit of incision defined as the lower edge of C2) with closure of the dura.
- Female patients of child bearing potential must agree to use contraception from the time of signing the informed consent form (ICF) until 90 days post-surgery.

Preoperative exclusion criteria for participants

- Female patients who are pregnant or breastfeeding.
- Assumed impaired coagulation due to medication or otherwise.
- Presence of infection.
- Any type of dural diseases in planned dural closure area.

- Patients requiring re-opening of planned surgical area within 90 days after surgery.
- Known allergy to any of the components of Liqoseal, DuraSeal or Adherus.
- Patients who previously received a Liqoseal, DuraSeal or Adherus.
- Patients who previously participated in this study or any investigational drug or device study within 30 days of screening.
- Presence of hydrocephalus (which will not be resolved by the surgical procedure).
- Patients with contra-indication to MRI.

Intraoperative inclusion criteria for participants

- Surgical wound classification Class I/Clean.
- Minimally 5 mm of dural space surrounding dural opening.

Intraoperative exclusion criteria for participants

- Patients in whom elevation of PEEP has a potential detrimental effect.
- Patients who will require a CSF drain, electrodes or other devices passing the dural layer or extra- to intracranial bypass surgery.
- Primary closure of the dura mater with material other than autologous material excluding fat.
- Patients in whom no intra-operative CSF leakage is present after primary closure of the dura mater with elevation of PEEP.
- A gap of > 3 mm after primary closure of the dura mater.
- Dural opening cannot be covered by Liqoseal (8x8 cm) with a 5-mm overlap.

Eligibility criteria for surgeons performing the intervention

- Only neurosurgeons and neurosurgical residents trained for the protocol and application of both the interventional and control device will perform the study interventions. Virtual training will be provided by the sponsor during the site initiation visit and online revision material is available throughout the study. Only trained and signed off surgeons can perform study actions. A delegate from the sponsor also attends the first surgery if wished for by the local center.

Who will take informed consent? {26a}

Informed consent will be collected by the principal investigator or designated study team member.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Participants will be asked to consent or not to the use of their data for other research related to the investigational device, to be contacted for future studies and to share video/photographs of the surgery with the sponsor of the study.

Interventions

Explanation for the choice of comparators {6b}

Investigational Device Description

The bioresorbable Liqoseal is indicated for use as an adjunct to standard methods of dural closure, such as suturing, to provide a watertight closure of the dura. Liqoseal consists of two layers; a watertight blue layer comprising biodegradable polyesterurethane and an adhesive white layer comprising biodegradable poly(DL-lactide-co-ε-caprolactone) copolymer and multiarmed N-hydroxylsuccinimide functionalized polyethylene glycol. This layer reacts with amines in the dural tissue in a moist environment, forming covalent bonds between the device and the tissue.

Control Device Description

The control arm of this study consists of two Food and Drug Administration (FDA) approved dural sealants for cranial use: DuraSeal and Adherus, indicated for use as an adjunct to standard methods of dural repair. Both consist of 2 components that when mixed together form an absorbable hydrogel. These products can be considered the current standard of care for dura sealing[6,7].

Intervention description {11a}

1. The patient is electronically randomized for Liqoseal or control device on the day of surgery by trained personnel other than the operating surgeon.
2. The assigned dural sealant is taken out of the freezer/storage and placed in a non-transparent box.
3. First PEEP test (for safety)
4. Dura mater is closed with the standard method of suturing.
5. Hemostasis should be achieved.
6. The dura mater surface is rinsed from particles with physiological saline and kept moist.
7. Second PEEP test (CSF leakage confirmed).
8. Surgeon is unblinded and the dural sealant is applied.
    a. For Liqoseal: the dry patch is cut into the required size, and positioned with. the white side against the sutured area of the dura mater and compressed for 2 minutes with a moist gauze.
    b. For Duraseal and Adherus: the hydrogels are applied aiming at the sutured area of the dura mater, holding the device 2-4 cm away, until a thin coating (1-2 mm) is formed.
9. Third PEEP test (2 minutes after device implantation).
10. Standard closure of cranial defect and soft tissue will then be undertaken per surgeon standard.

Criteria for discontinuing or modifying allocated interventions {11b}

Once the patient is allocated to the control or interventional arm the patient can still be excluded from the study in case the intraoperative eligibility criteria are not met or in case the first PEEP test was not considered safe for the patient. Once the allocated intervention has been applied this can be modified in case the patient reaches the primary outcome during the surgery (intraoperative CSF leakage at the third PEEP test). In such case the neurosurgeon can undertake any actions deemed necessary to ensure optimal patient care in the situation.

Strategies to improve adherence to interventions {11c}

There is no patient action required to adhere to the intervention protocol. Surgeons are blinded until after primary closure of the dura to optimize adherence to the interventions independent of the allocated intervention.

Relevant concomitant care permitted or prohibited during the trial {11d}

Participants are not allowed to participate in any other investigational drug or device study. All other forms of treatment are permitted.

Provisions for post-trial care {30}

Insurance is in place, to enable compensation in the event of an injury to a participant.

Outcomes {12}

Primary Endpoint

The primary endpoint is a composite endpoint defined as the occurrence of any of the following within 90 days of surgery:

- Wound infection defined in accordance with the Centers for Disease Control and Prevention guidelines as deep incisional (cat II) or organ or space infection (cat III).(8)
- Intraoperative CSF leakage after device application at a positive end expiratory pressure (PEEP) of 20 cm H_2O.
- Percutaneous CSF leakage confirmed by β-2 transferrin test.
- Pseudomeningocele requiring puncture, external lumbar drainage or surgical re-intervention.
- Pseudomeningocele >20 cc as confirmed on MRI.

Secondary Endpoints

Safety

- Device related adverse events (AEs) and serious adverse events (SAEs) throughout the study up to 90 days after surgery.

- Complications requiring surgical re-intervention up to 90 days after surgery.

Performance

- Any pseudomeningocele as confirmed on magnetic resonance imaging (MRI) at day 90.
- Volume of pseudomeningocele as determined on MRI at day 90.
- Ease of use and application of the Liqoseal.

Participant timeline {13}

Screening will take place between day 90 and day 1 prior to surgery **(figure 2 and figure 3)**. Follow-up will take place at day 7 or discharge (whichever comes first), day 30 and day 90.

During hospitalization, the patient will be monitored daily for clinical signs of infection and CSF leakage or swelling at the surgical wound. All patients will undergo an MRI on day 90.

	SCREENING	PROCEDURE	FOLLOW-UP		
	Day -90 to Day 1	Day 1	Discharge or Day 7 [a, b]	Day 30 [b] *(± 5 days) [i]*	Day 90 [b] *(± 14 days) [i]*
Informed consent	X				
Demographics	X				
Comorbidity, Medical / Surgical History	X				
Preoperative Eligibility Check	X				
Physical Exam	X				
Pregnancy test (female subjects only) [c]	X				
Randomization		X			
Surgery		X			
PEEP [d]		X			
Intraoperative Eligibility Check		X			
Device application		X			
Photograph of surgical site [e]		X			
Inspection wound / clinical signs of infection [f]		Continuously monitored during hospitalization		X	X
β-2 transferrin test [g]			X	X	X
Blood samples [h]			X	X	X
MRI					X
Adverse Events		X	X	X	X
Concomitant Medication	X	X	X	X	X
User Experience and Device Deficiency		X			

a) Discharge or Day 7 (± 1 day) (whichever comes first).
b) Follow-up based on day of Procedure (Day 1).
c) For female childbearing potential subjects only, a pregnancy test will be performed within 48 hours before procedure (urine or blood test).
d) If no spontaneous leakage of CSF after sutured closure of dura, PEEP elevation will be performed until CSF leakage occurs. After application of the device, this elevation will also be performed.
e) Photo to be taken, after sutured closure of dura, pre- and post-application of the device.
f) During hospitalization, inspection of wound will be monitored continuously as well as signs of infection. Data will be collected every 24-hours from 24 hours after surgery until Discharge or Day 7 (whichever comes first). Also on Day 30 and Day 90 this data will be collected.
g) Only if external wound leakage is visible or a pocket puncture is performed, β-2 transferrin test will be performed.
h) Blood samples will be taken for CRP and leucocytes only in case of clinical signs of infection.
i) Extension of the window if necessary due to restrictions in regards to COVID-19. Window will be for Day 30 -5, +10 and for Day 90 +/- 20 days.

Figure 2. Spirit figure: schedule of enrolment, interventions and assessments.

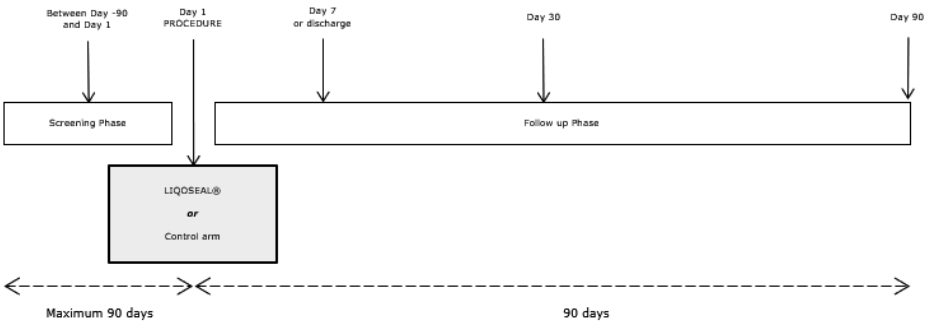

Figure 3. Study Scheme.

Sample size {14}

'Success' was defined as absence of the composite endpoint. The success rate of the investigational product was set similar to that of the control devices combined. The combined success rate of the control devices was determined at 91%, based upon a comparative study of the two devices[6]. The non-inferiority margin was set at 10%, this was accordance with the design used by the control devices[6] and believed to be clinically acceptable. The power of the study is 80%, with a one-sided significance level of 5%. The expected rate of attrition is 10%. Under these assumptions, a total of 228 patients are required, 114 per arm.

Recruitment {15}

Prior to the start of the trial estimations of recruitment numbers were provided by all participating sites. Recruitment is currently ongoing. Due to the impact of the covid-19 crisis the recruitment time has been extended to 12 months.

Assignment of interventions: allocation

Sequence generation {16a}

The randomization schedule will be generated using an internet-based computerized randomization program within the research management platform (RMP) and electronic data capturing system Staicy v2.33 (IQVIA MedTech, Danbury, USA). The RMP is fully validated and 21 CFR part 11 (and EU Annex 11) compliant. It is developed under an ISO27001 certified quality management system.

Patients will be stratified by study site and type of cranial surgery (craniotomy or craniectomy) in blocks of 4 and randomized (1:1) at the start of surgery.

Concealment mechanism {16b}

Concealment of the randomization scheme is ensured by use of the RMP which shows the user the assigned randomization per individual patient for their site only.

The assigned device will be stored in a non-transparent box directly after randomization.

Implementation {16c}

Randomization is performed by trained personnel other than the operating surgeon by logging into the RMP to perform randomization for each individual patient at the time.

Assignment of interventions: Blinding

Who will be blinded {17a}

The surgeon is blinded for group allocation until finalization of the primary closure of the dura, by concealing the allocated device in a non-transparent box.

Procedure for unblinding if needed {17b}

The surgeon is unblinded after finalization of the primary closure of the dura, to apply the allocated device.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Preoperative data

Demographic information (i.e., gender, childbearing potential, age, length, weight and body mass index), medical and surgical history (i.e., indication for surgery, allergies, tobacco use, medication use), as well as comorbidity of the patient will be collected. A physical exam is performed during screening. All female patients of child-bearing age will undergo a pregnancy test.

Intraoperative data

The device used and its size, LOT number, size of trepanation, any use of autologous material, and type of suture are recorded.

To determine the intra-operative CSF leak before and after the application of the device, the PEEP will be increased to 20 cmH2O for 20 seconds. First, this test will be performed before closure of the dura to determine safety for the postoperative intracranial field (control of hemorrhage, swelling or other potential adverse effects). Upon completion of the primary sutured dural closure and before the application of the sealant, the closure of the dura will be evaluated for CSF leakage by repeating the test. The patient

is excluded if there is no leakage at PEEP of 20 cmH2O for 20 seconds. Two minutes after application of the device, the test will be performed for a third time to evaluate CSF leakage. All 3 PEEP elevations and application of the device will be recorded on video. A photograph will be taken before and after device application.

After the procedure, end users (surgeons, scrub nurses) will be invited to complete several closed-end questions regarding their user experience with Liqoseal.

Postoperative data
During the hospitalization, the subject will be monitored daily for clinical signs of infection. The surgical wound will be inspected daily starting 24 hours after surgery. Blood analysis and a wound culture will be performed if there are clinical signs of infection. In case of CSF leakage from the incision, a β-2 transferrin test will be performed. Data will be collected every 24-hours from 24 hours after surgery until discharge or Day 7 (whichever comes first).

The clinical data to be collected on the e-CRF includes the following: Body temperature.

In case of signs of infection the following data will be collected as well: C-Reactive Protein (CRP), leucocytes, culture of wound.

The signs of infection will be classified to the Centers for Disease Control and Prevention (CDC) standard of Surgical Site Infection (SSI) and recorded in the e-CRF[8].

All subjects will undergo an MRI on Day 90. The MRI will be performed to collect data on the presence and amount of pseudomeningocele (any subcutaneous fluid on T2) as well as the long-term thickness of dura mater and investigational device.

Independent radiologists will analyze the MRIs of all subjects for the outcome measurements. Each MRI will be evaluated by 3 independent radiologists, whereas the analysis then will be based on minimally 2 out of 3 evaluations (whom are in consensus).

Postoperative assessments are not blinded.

Plans to promote participant retention and complete follow-up {18b}
Participants will be contacted through telephone, after 3 unsuccessful attempts to reach the subject, a registered mail will be sent to the subject to indicate the need for a follow-up appointment. If these communications are unsuccessful, the subject will be considered lost to follow-up.

Data of withdrawn subjects, collected up until the point of withdrawal, will be preserved and used in the applicable analyses. The reasons for withdrawal will be compared between treatment arms, to assess potential bias in the analysis. The reason

for discontinuation must be recorded in the source documentation and the e-CRF. Possible reasons for discontinuation of participation may include, but are not limited to, the following reasons:

- subject decides to withdraw from the study;
- adverse events;
- lost to follow-up

Data management {19}

An electronic data capturing (EDC) system will be used to collect data on a secure, internet-based electronic case report form (e-CRF), and image transfer software. The principal investigator (PI) or his/her designee at the clinical site will perform primary data collection by entering the data into the e-CRF. Only the PI or other predesignated personnel will be authorized to enter data using their unique login credentials. Each user access to the system will be tracked so that all data operations can be monitored and verified.

The e-CRF will be completed on a continuous basis starting from the point of enrollment to final follow-up.

A critical quality control shall be performed for the first 2 subjects by the sponsor's designated data management team and queries issued where needed. Such queries must be reviewed by the monitor prior to alerting the site personnel to answer them.

After the monitor has done the source document verification and obtained satisfactory answers to eventual queries from the site, a full quality control shall be performed on the monitored data throughout the clinical investigation by the designated data management team and queries issued where needed. This process will be repeated till the end of the clinical investigation so as to allow for a timeline freezing of the data base for statistical analysis.

Confidentiality {27}

The investigator must ensure that subjects' anonymity will be maintained. On e-CRFs or other documents submitted to the sponsor, subjects should not be identified by their names, but by the subject number. The investigator must keep a subject identification code list showing the enrolment number, the subject's name, date of birth and address or any other locally accepted identifiers. All information to be sent to the sponsor concerning patients and their participation in the study will be considered confidential. All data will be processed without identifiable reference to the individual patient.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}
The collection and evaluation of laboratory tests will be performed according to the standard procedures per study site. The following laboratory tests may be applicable to participants in this this trial: pregnancy test, CRP, leucocytes, β-2 transferrin test.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}
The primary analysis set will consist of a modified intention to treat analysis (mITT). Modified intent-to-treat (mITT) analysis set is a subset of ITT analysis set and will consist of all enrolled subjects (subjects who have signed the ICF, meet all inclusion criteria, meet none of the exclusion criteria and the investigational/control device has contacted the subject's dura) with evaluable data for the primary endpoint.

The primary endpoint will be evaluated for statistical significance based on the Wald method for difference of proportions. If non-inferiority is met a one-sided significance level of 5%, the difference in success rates will also be evaluated for superiority at a one-sided significance level of 5%.

The primary endpoint will also be summarized by investigational site and cranial procedure type. Interactions of treatment and site/procedure type may be examined graphically or using logistic regression with success rate at day 90 as the response variable, and the study center (or procedure type), treatment group, study center (or procedure type) by treatment group interaction as predictor variables in the model. If the results of the test show evidence of heterogeneity across sites/procedure types (i.e., p-value<0.15), then the treatment/procedure type effect will be evaluated further to identify any confounding factors.

All safety analyses will be based on subjects in the intention-to-treat (ITT) analysis set. The ITT analysis set will consist of all enrolled subjects (subjects who have signed the ICF, meet all inclusion criteria, meet none of the exclusion criteria, the investigational/control device has contacted the subject's dura). Results based on the ITT analysis set will be analyzed according to each subject's randomization assignment.

Interim analyses {21b}
There will be a safety stop after enrolment of 50 patients in the USA per the FDA. The 90-day safety data of the first 30 patients, will be provided to the FDA to request approval to complete enrolment.

Methods for additional analyses (e.g. subgroup analyses) {20b}
As a supplementary analysis, the primary endpoint will be evaluated in a per protocol analysis (excluding subjects with protocol deviations) and ITT analysis. No supplementary of sensitivity analyses are planned for the safety and secondary endpoints.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}
By definition, the primary analysis of primary endpoint will be based on available data only, and no imputation for missing data will be performed. The supplementary analysis of primary endpoint based on the ITT analysis set will likely include some subjects who are excluded from the mITT analysis set and do not have evaluable data for the primary endpoint. Subjects who die prior to reaching the 90-day primary endpoint without experiencing any of the primary endpoint outcomes will be excluded from analysis. Data for any other subjects that do not have evaluable data at day 90 will be imputed using multiple imputation in the ITT analysis. The analysis of secondary endpoints will be based on available data only, with no imputation for missing data. Subjects who die or withdraw from the study for other reasons prior to experiencing a specific adverse event will be included in the denominator and assumed to have not experienced the event.

Plans to give access to the full protocol, participant level-data and statistical code {31c}
The full protocol will not be published. An abbreviated protocol has been registered at Clinicaltrials.gov (number NCT04086550) prior to study initiation. The participant-level dataset and statistical code will not be granted for public access.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}
There are two coordinating investigators for this trial; one from the USA and one from Europe. The study will be managed by a project manager and study coordinator employed by the sponsor. The Sponsor' study team, a PhD student and both coordinating investigators will meet at a weekly basis. Day-to-day performance of the trial at the sites will be performed by site personnel trained for the study. Contact with the sites between Sponsor, coordinating investigators and sites' study personnel will be at a regular basis, dependent on the need for the site based upon enrolment rate and site proficiency. The day-to-day performance of the trial by the sites will be supported and monitored by an independent CRO, who will be in contact with the Sponsor, coordinating investigators and sites' study staff at a regular basis based on the need for the site based upon

enrolment rate and site proficiency. An independent data monitoring committee (DMC) will be appointed consisting of at least 3 specialists in the field of neurosurgery, who will assess patients' safety and trial progress based upon enrolment rate and (Serious) Adverse Events on a regular basis.

Composition of the data monitoring committee, its role and reporting structure {21a}

The data monitoring committee (DMC), consisting of at least 3 specialists in the field of neurosurgery, will review data relating to safety and performance and to ensure the continued scientific validity and merit of the study. Further details regarding the DMC can be found in its charter (Supplementary Material II). Following each meeting a formal report will be prepared, which will be sent to the sponsor after approval of all members of the DMC. The DMC is independent from the sponsor, and members have no competing interests.

Adverse event reporting and harms {22}

During the study, (serious) adverse events and (serious) anticipated and unexpected adverse device effects will be recorded; reporting will be done from point of enrolment till end of study.

The (principal) investigators shall report all adverse events and device deficiencies in the appropriate sections of the e-CRF and provide where requested by the sponsor, the necessary clinical or technical information that may contribute to clarifying the circumstances.

The (principal) investigators shall report all serious adverse events (SAEs) and device deficiencies (DDs) that might have led to a SAE: if a) suitable action had not been taken or b) intervention had not been made or c) if circumstances had been less fortunate, and new findings/updates in relation to already reported events to the sponsor and record in the e-CRF within 24 hours after awareness of the event.

Any other reportable events as described above or a new finding/update to a reported event shall be reported immediately, but not later than 7 calendar days following the date of awareness by the sponsor of the new reportable event or of new information in relation with an already reported event.

Frequency and plans for auditing trial conduct {23}

Sponsor monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of the main safety and performance endpoints. Additional checks of the

consistency of the source data with the e-CRFs are performed according to the study-specific monitoring plan. An initiation visit will be performed before the first subject is enrolled. The first one site monitoring visit will take place within 21 calendar days of the first patient randomized. Further visits will take place at least twice a year. A risk-based monitoring approach will be utilized and the data points that are source data verified as well as the frequency of monitoring visits will be based upon enrollment, data integrity, and site compliance

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

No changes in the clinical investigation procedures shall be effected without mutual agreement of the principal investigator and the sponsor. The agreement of the changes must be documented by signing the corresponding clinical investigation plan amendments. All changes require notification to the EC/IRB and the Competent Authority/FDA (when appropriate).

Dissemination plans {31a}

Within one year after the end of the study, a final study report with the results of the study, including any publications/abstracts of the study, will be submitted to the local ethics committee/institutional review board and the applicable competent authorities. Furthermore, the results of the study will be published in a scientific publication. If requested the results of the study will be shared with participants.

Discussion

This is the first randomized controlled trial in which the safety and efficacy of Liqoseal will be compared to the best current practice. This trial will evaluate whether Liqoseal is non-inferior to control as a means of reducing CSF leakage and safety.

Trial status

Recruiting. First patient enrolled May 20th 2021.

Abbreviations

CSF: cerebrospinal fluid
e-CRF: electronic case report form
EDC: electronic data capturing
ICF: informed consent form
ITT: intention to treat
mITT: modified intention to treat

PEEP: positive end expiratory pressure
PI: principal investigator
RMP: research management platform

Acknowledgements
We want to thank all the authors of the ENCASE II study group*: E.H.J. Voormolen, J.W. Dankbaar, P. Depauw, B. Brouwers, M.R. Germans, E. Baert, J. Vandersteene, C.F. Freyschlag, J. Kerschbaumer, C. Thomé, F. Zenga, F. Penner, A. Abdulazim, M. Sabel, M. Rapp, T. Beez, M. Zuccarello, E. Sauvageau, K. Abdullah, B. Welch, D. Langer, J. Ellis, A. Dehdashti, J. Van Gompel, B. Bendok, K. Chaichana, J. Liu, A. Dogan, M.K. Lim, M.G. Hayden for their valuable contribution to this trial.

Authors' contributions {31b}
APC is the coordinating investigator globally, TPCvD is the coordinating investigator in Europe, and both coordinating investigators conceived the study, led the proposal and protocol development. EMHS drafted the first version of the manuscript and contributed to the development of the protocol. All authors read and approved the final manuscript.

Funding {4}
This study is funded by Polyganics BV, Rozenburglaan 15A, 9727 DL Groningen, The Netherlands.

Availability of data and materials {29}
The final dataset and the resulting study report will be included in the Trial Master File for the study. This will be archived at the Sponsor and the participating sites. Only dedicated personnel at the Sponsor and the participating sites will have access to the files. The files will be requested to be archived for 15 years after study conclusion.

Ethics approval and consent to participate {24}
This study is performed in accordance with the World Medical Association Declaration of Helsinki[9], ISO 14155:202011[10], the Medical Device Directive (MDD 93/42/EEC and MEDDEV 2.7/3 rev. 3:2015)[11] and MEDDEV 2.7/413[12], FDA regulations for IDE studies (G200118/S002) including 21 CFR 50, 54, 56, and 81214, and the Health Insurance Portability and Accountability Act[13]. The protocol was approved by the local ethics committees or institutional review boards of all centers that are currently recruiting: Ethics Committee of Citta' Della Salute e Della Scienza di Torino #153/2021, University Hospital Zurich #BASEC 2021-00407, Ghent University Hospital #B6702021000353, University Medical Center Utrecht #NL71524.041.19, Institutional Review Board of University of New Mexico #21-173, University of Texas Southwestern #STU-2019-

1127, University of Cincinnati #2021-0336, Oregon Health and Science University #STUDY00023102

Written, informed consent to participate will be obtained from all participants.

Consent for publication {32}

Not applicable.

Competing interests {28}

TPCvD received a consultancy fee in the design phase of the product from Polyganics BV.
EMHS receives a research grant through Polygancis BV.
APC received no financial support from Polyganics BV.

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Appendix I: list of participating sites

University of New Mexico's Health and Sciences Center, Albuquerque, USA
Lennox Hill / Northwell, New York, USA
University of Texas Southwestern Medical Center, Dallas, USA
University of Cincinnati Health, Cincinnati, USA
Oregon Health & Science University, Portland, USA
Mayo Clinic, Jacksonville, USA
Mayo Clinic, Rochester, USA
Stanford Health Center, Stanford, USA
University Medical Center Utrecht, Utrecht, the Netherlands
Elisabeth-TweeSteden Ziekenhuis, Tilburg, the Netherlands
University Hospital Zurich, Zurich, Switzerland
Citta della Salute, Torino, Italy
Mannheim University Hospital, Mannheim, Germany
University Hospital Dusseldorf, Dusseldorf, Germany
University Hospital Ghent, Ghent, Belgium
University Hospital Innsbruck, Innsbruck, Austria

II

Safety and efficacy of Dura Sealant Patch in transsphenoidal surgery


4

Cerebrospinal fluid leak after transsphenoidal surgery – A systematic review and meta-analysis

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Journal of Neurological Surgery Skull Base. 2021. 20;83(Suppl 2):e501-e513
doi:10.1055/s-0041-1733918.

Abstract

Background

Cerebrospinal fluid (CSF) leak is widely recognized as a challenging and commonly occurring postoperative complication of transsphenoidal surgery (TSS).

The primary objective of this study is to benchmark the current prevalence of CSF leak after transsphenoidal surgery in the adult population.

Methods

The authors followed the PRISMA guidelines. The PubMed, Embase and Cochrane Library databases were searched for articles reporting CSF leak after transsphenoidal surgery in the adult population. Meta-analysis was performed using the Untransformed Proportion metric in OpenMetaAnalyst. For two between-group comparisons a generalized linear mixed model was applied.

Results

We identified 2,408 articles through the database search, of which 70, published since 2015, were included in this systematic review. These studies yielded 24,979 patients who underwent a total of 25,034 transsphenoidal surgeries. The overall prevalence of postoperative CSF leak was 3.4% (95% CI 2.8-4.0%). The prevalence of CSF leak found in patients undergoing pituitary adenoma resection was 3.2% (95% CI 2.5-4.2%), whereas patients who underwent TSS for another indication had a CSF leak prevalence rate of 7.1% (95% CI 3.0-15.7%) (OR 2.3, 95% CI 0.9-5.7). Patients with cavernous sinus invasion (OR 3.0, 95% CI 1.1-8.7) and intraoperative CSF leak (OR 5.9, 95% CI 3.8-9.0) have increased risk of postoperative CSF leak. Previous transsphenoidal surgery and microscopic surgery are not significantly associated with postoperative CSF leak.

Conclusion

The overall recent prevalence of CSF leak after TSS in adults is 3.4%. Intraoperative CSF leak and cavernous sinus invasion appear to be significant risk factors for postoperative CSF leak.

Key words

Complications; CSF leak; Endonasal; Liquorrhea; Pituitary Adenoma Surgery; Skull Base

Introduction

Cerebrospinal fluid (CSF) leak is still widely recognized as a commonly occurring postoperative complication of transsphenoidal surgery (TSS). CSF leak is associated with various complications including meningitis, intracranial infection and CSF hypotension syndrome. These complications often lead to additional healthcare costs and substantial morbidity as they may require prolonged hospitalization, reoperation and external lumbar drainage (ELD)[1,2]. Grotenhuis (2005) reports an additional cost of €10.243 per patient with postoperative CSF leak for transsphenoidal procedures[2]. The prevalence of postoperative CSF leak seems increased in patients with an elevated body mass index (BMI) and/or increased intracranial pressure[3]. However, the exact risk of this complication and variables of influence are not clearly defined and reported prevalence rates vary widely (0-40%)[4]. CSF leak rates among patients undergoing TSS are regarded to be higher than for transcranial neurosurgical procedures due to additional risk factors, such as gravity and a lack of anatomical barriers provided by watertight dural closure and subcutaneous and cutaneous closure[5]. However, techniques of closure have been significantly improved by using a vital nasoseptal mucosal flap, the use of sealing materials and improved neurosurgical techniques[6-9]. Transsphenoidal surgery has been an evolving field over the last decades, therefore complication rates should be investigated in recent literature and frequently updated as advancements in the surgical technique continue. The objectives of this study are to benchmark the prevalence of CSF leak after transsphenoidal surgery in the adult population in the past 5 years, and to define variables affecting this risk.

Methods

The authors followed the PRISMA guidelines for this systematic review and meta-analysis[10].

Search strategy and study selection

We performed a literature search in the PubMed, Embase and Cochrane Library databases for articles reporting CSF leak after transsphenoidal surgery until April 1, 2020. A combination of free, controlled and Mesh/Emtree terms for transsphenoidal surgery and CSF leak, such as “Transsphenoidal” OR “Endoscopic endonasal” AND ““Cerebrospinal fluid leak” OR “Cerebrospinal fluid rhinorrhea”, were used to form a search string (see **Appendix A-C**, for the search strings per database).

Articles reporting original studies published since 2015 on the adult population reporting CSF leak rates after TSS written in English or Dutch were included. The timeframe 2015-2020 was chosen with the aim to provide an up-to-date analysis of CSF leak after TSS and to expand on the existing literature on this topic[4]. Extended

procedures and use of dural sealants were no restriction for inclusion. Studies including CSF fistula repairs or biopsies were excluded. Furthermore, case reports (n<30) were excluded, as these were not considered strong evidence due to the risk of publication bias and selected populations.

Two authors (R.S. and E.M.H.S.) independently screened titles and abstracts for eligibility, after which full-texts of all potentially eligible studies were assessed for inclusion. No disagreement regarding the inclusion of an article after full-text assessment was encountered. The reference lists of all included studies and relevant reviews were cross-checked for additional eligible articles.

Data collection

We extracted the following data from the included studies: study characteristics (authors, publication year, inclusion period, design, country, center name, total number of patients, total number of surgeries); patient characteristics (mean age at surgery, number of females, mean BMI, mean follow up duration, previous surgery at same site, type of sphenoid sinus, preoperative diabetes mellitus, use of immunosuppressive medication, use of blood thinners, preoperative hydrocephalus, preoperative pneumocephalus, history of skull base radiation, length of stay); surgery characteristics (indication (e.g. pituitary or craniopharyngioma resection), approach, extended or conventional (based on the article's definition), reconstruction technique, use of sealant, intraoperative placement of a CSF diversion shunt); tumor characteristics (type of tumor, maximal tumor diameter, invasive (Knosp grades 3 and 4) or not, suprasellar extension); outcome parameters (rate of intraoperative CSF leak and rate of postoperative CSF leak, as defined by the article). Studies with a non-comparative design were defined as case series[11]. The study quality of case series was assessed using the National Heart, Lung and Blood Institute of National Institutes of Health (NIH) quality assessment tool for case series studies[12], whereas the Newcastle Ottawa Scale[13] was used for the quality assessment of cohort studies. Studies with fewer than six points were judged to be of poor quality, studies with six or seven points were deemed of fair quality and studies with more than seven points were classified as being of good quality. Each item was awarded one point if answered with 'Yes' or a star.

Statistical analysis

We performed a meta-analysis of prevalence using the Untransformed Proportion metric in OpenMetaAnalyst for Sierra, version 10.12. A binary random effects analysis using the DerSimonian-Laird method was applied if heterogeneity across studies was significant ($p<0.05$). For non-significant heterogeneity across studies the binary fixed effects inverse variance model was used. For two between-group comparisons

(microscopic vs. endoscopic surgery and pituitary adenoma resection vs. other indication) a generalized linear mixed model was applied, using SAS version 9.4 (SAS Institute Inc), as these analyses involved comparisons of groups on study level. Heterogeneity across studies was ascertained through Higgins I^2 [14].

The prevalence of CSF leak after transsphenoidal surgery with 95% confidence interval (CI) was the primary outcome measure in this study. For between group comparisons of patients with and without certain risk factors for CSF leak the outcome measures were odds ratios (OR) with 95% CI. We performed three sensitivity analyses 1. excluding Pines et al.[32], as this publication accounts for almost half of the total population, 2. a comparison between studies published between 2015-2017 and 2018-2020 to evaluate a learning curve, 3. high quality studies only.

Results

Included studies

We identified 2,408 articles through the initial database searches after removing duplicates. Seventy articles met the inclusion criteria for this systematic review. Eight articles were excluded from the meta-analysis due to an overlapping population with another included article (the study with the largest sample size was included)[15-22]. One article was manually added by hand-searching the reference lists of all included articles. The study selection process and reasons for exclusion are shown in **Figure 1.**

The included studies yielded 24,979 patients who underwent a total of 25,034 transsphenoidal surgeries as some subjects had more than one surgery. This includes 262 extended procedures and 2,104 conventional procedures. In the remaining 58 articles insufficient information is provided to determine the number of extended and conventional surgeries. An overview of study characteristics is presented in **Table 1**. Nineteen studies were judged to be of good quality, 37 studies of fair quality and 14 studies of poor quality (see **Supplementary Material 1**, for an overview of quality assessment).

There was insufficient data from the included studies to perform reliable analyses for a number of risk factors: suprasellar extension, dural invasion, BMI, preventative external lumbar drainage, reconstruction technique, age at surgery, sex, diabetes mellitus, use of immunosuppressive medication, use of blood thinners, preoperative hydrocephalus, preoperative pneumocephalus, history of skull base radiation and sealant use.

Outcome and risk factor analysis

The overall prevalence of postoperative CSF leak was 3.4% (95% CI 2.8-4.0%) (**Figure 2**). Heterogeneity across studies was substantial (I^2 81.7).

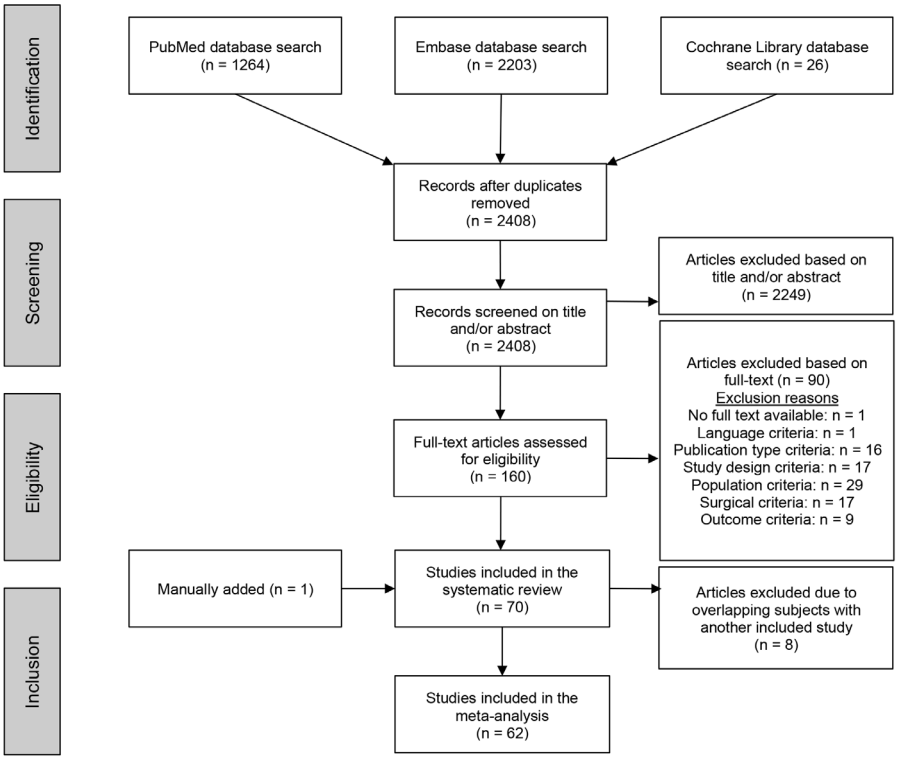

Figure 1. Flowchart demonstrating the study selection process

Table 1. Overview of study characteristics of included studies.

Authors	Year	Study design	Approach	Indication	Population (N=24,979)	Surgeries (N=25,034)	CSF leak (%)	Study quality
Gondim et al.[23]	2015	RC	Endoscopic	Pituitary tumor	374	374	3.7	Good
Fathalla et al.[24]	2015	RC	Endoscopic and Microscopic	Pituitary tumor	65	65	6.2	Good
Wang et al.[21, a]	2015	CS	Endoscopic	Pituitary tumor	1166	1166	0.6	Fair
Nie et al.[25]	2015	CS	Endoscopic	Pituitary tumor	52	52	0.0	Fair
Zhan et al.[26]	2015	RC	Endoscopic	Pituitary tumor	313	318	3.8	Fair
Ishii et al.[27]	2015	RC	Endoscopic	Pituitary tumor, craniopharyngioma, meningioma, chordoma, ependymoma	48	48	6.3	Poor
Park et al.[28]	2015	CS	Endoscopic	Pituitary tumor, Rathke's cleft cyst, craniopharyngioma, meningioma, chordoma/ chondrosarcoma, other	188	197	0.0	Poor
Chabot et al.[29]	2015	CS	Endoscopic	Pituitary tumor	39	39	10.3	Fair
Pinar et al.[30]	2015	CS	Endoscopic	Pituitary tumor	32	32	9.4	Fair
Sanders-Taylor et al.[31]	2015	RC	Endoscopic	Pituitary tumor	264	264	1.9	Poor
Pines et al.[32]	2015	RC	Unknown	Pituitary tumor	12938	12938	1.7	Poor
Xie et al.[33]	2016	RC	Endoscopic	Pituitary tumor	43	43	14.0	Good
Gao et al.[34]	2016	RC	Endoscopic and Microscopic	Pituitary tumor	105	105	10.5	Fair
Freyschlag et al.[35]	2016	CS	Endoscopic and Microscopic	Pituitary tumor	50	50	0.0	Fair
Park, Hong et al.[36]	2016	RC	Endoscopic	Pituitary tumor, craniopharyngioma, meningioma, chordoma, Rathke's cleft cyst, other	106	106	9.4	Fair
Jang et al.[37]	2016	CS	Endoscopic	Pituitary tumor	331	331	1.8	Good
Zaidi et al.[38]	2016	RC	Endoscopic and Microscopic	Pituitary tumor	135	135	1.5	Fair
Gondim, Albuquerque et al.[17, b]	2017	CS	Endoscopic	Pituitary apoplexy	39	39	0.0	Poor
Fnais et al.[39]	2017	RC	Endoscopic	Pituitary tumor, pituitary apoplexy, Rathke's cleft cyst, craniopharyngioma, other	145	138	11.6	Fair

Table 1. *(continued)*

Authors	Year	Study design	Approach	Indication	Population (N=24,979)	Surgeries (N=25,034)	CSF leak (%)	Study quality
Ye et al.[40]	2017	RC	Endoscopic	Pituitary tumor	1281	1281	0.5	Poor
Karki et al.[41]	2017	RC	Microscopic	Pituitary tumor	123	123	15.4	Good
Wang, Guo et al.[42]	2017	RC	Microscopic	Pituitary tumor	51	51	0.0	Good
Sun et al.[43]	2017	CS	Endoscopic	Pituitary tumor	42	42	9.5	Poor
Ding et al.[44]	2017	RC	Endoscopic	Craniopharyngioma	33	33	18.2	Good
Zhou et al.[45]	2017	RC	Endoscopic	Pituitary tumor	492	492	1.2	Fair
Cebula et al.[46]	2017	PC	Endoscopic	Pituitary tumor	230	230	0.0	Fair
Levi et al.[47]	2017	RC	Endoscopic and Microscopic	Pituitary tumor	221	221	5.9	Poor
Zoli et al.[48]	2017	CS	Endoscopic	Pituitary tumor	75	75	1.3	Fair
Fujimoto et al.[49]	2017	RC	Endoscopic	Pituitary tumor	161	162	4.9	Poor
Yano et al.[22, c]	2017	CS	Endoscopic	Pituitary tumor	32	34	5.9	Fair
Sasagawa et al.[50]	2017	RC	Endoscopic and Microscopic	Pituitary tumor	78	78	1.3	Fair
Fishpool et al.[51]	2017	CS	Endoscopic	Pituitary tumor	32	32	0.0	Poor
Ajlan et al.[52]	2017	RC	Endoscopic	Pituitary tumor	176	176	4.5	Fair
Przybylowski et al.[53]	2017	RC	Endoscopic	Pituitary tumor	96	96	4.2	Good
Negm et al.[18, d]	2017	PC	Endoscopic	Pituitary tumor	41	41	2.4	Good
Shin et al.[54]	2017	CS	Endoscopic	Pituitary tumor	50	50	4.0	Fair
Patel et al.[3]	2018	RC	Endoscopic	Pituitary tumor, Rathke's cleft cyst, craniopharyngioma, other	806	806	4.7	Fair
Eseonu et al.[55]	2018	CS	Endoscopic	Pituitary tumor	275	275	3.6	Good
Popov et al.[56]	2018	RC	Endoscopic and Microscopic	Pituitary tumor	128	128	3.9	Fair

Table 1. *(continued)*

Authors	Year	Study design	Approach	Indication	Population (N=24,979)	Surgeries (N=25,034)	CSF leak (%)	Study quality
Hansasuta et al.[57]	2018	PC	Endoscopic	Pituitary tumor	183	220	3.6	Fair
Han et al.[58]	2018	CS	Endoscopic	Pituitary tumor	52	52	3.8	Good
Guo et al.[59]	2018	RC	Unknown	Pituitary tumor	53	53	9.4	Fair
Schuss et al.[60]	2018	RC	Endoscopic and Microscopic	Pituitary tumor	255	255	6.7	Poor
Hajdari et al.[61]	2018	CS	Endoscopic	Pituitary tumor	170	170	8.2	Fair
Karamouzis et al.[62]	2018	CS	Endoscopic	Pituitary tumor	90	90	4.4	Fair
Lofrese et al.[63]	2018	RC	Endoscopic	Pituitary tumor	95	95	5.3	Fair
Cudal et al.[64]	2018	CS	Unknown	Pituitary tumor, other	47	47	6.4	Poor
Robins et al.[65]	2018	RC	Endoscopic	Pituitary tumor	142	142	0.7	Poor
Barger et al.[66]	2018	CS	Endoscopic	Pituitary tumor	43	43	2.3	Good
Wilson et al.[67]	2018	RC	Endoscopic	Pituitary tumor	135	135	0.0	Good
Rehman et al.[68]	2018	CS	Endoscopic	Pituitary tumor	63	63	15.9	Fair
Xue et al.[69]	2019	RC	Endoscopic	Pituitary apoplexy	79	79	12.7	Fair
Chen et al.[15, e]	2019	CS	Endoscopic	Pituitary tumor	79	79	5.1	Fair
Fallah et al.[70]	2019	CS	Endoscopic	Pituitary tumor	80	88	4.5	Good
Spina et al.[71]	2019	RC	Unknown	Pituitary tumor	336	336	0.6	Good
Shen et al.[72]	2019	CS	Endoscopic	Pituitary tumor	45	45	2.2	Fair
Eichberg et al.[16, f]	2019	CS	Endoscopic	Pituitary tumor	120	120	1.7	Fair
Chen, Sprau et al.[73]	2019	RC	Endoscopic	Pituitary tumor	131	131	8.4	Good
Seltzer et al.[20, g]	2019	CS	Endoscopic and Microscopic	Pituitary tumor	52	52	1.9	Fair
Azab et al.[74]	2019	RC	Microscopic	Pituitary tumor	205	205	2.9	Good
Memel et al.[75]	2019	RC	Unknown	Pituitary tumor	115	115	2.6	Fair

Table 1. *(continued)*

Authors	Year	Study design	Approach	Indication	Population (N=24,979)	Surgeries (N=25,034)	CSF leak (%)	Study quality
Rieley et al.[76]	2020	RC	Endoscopic	Pituitary tumor, other	427	427	13.1	Poor
Liu et al.[77]	2020	RC	Endoscopic	Pituitary tumor	189	189	6.3	Fair
Zhang et al.[78]	2020	CS	Endoscopic	Pituitary tumor	113	113	0.9	Fair
Tardivo et al.[79]	2020	RC	Endoscopic	Pituitary tumor	81	81	4.9	Good
Castaño-Leon et al.[80]	2020	RC	Endoscopic and Microscopic	Pituitary tumor, other	187	187	5.3	Fair
Pangal et al.[19, g]	2020	CS	Endoscopic	Pituitary apoplexy	50	50	8.0	Fair
Parikh et al.[81]	2020	CS	Endoscopic	Pituitary tumor	334	334	3.9	Good
Tafreshi et al.[82]	2020	CS	Endoscopic	Pituitary tumor, Rathke's cleft cyst, arachnoid cyst, xanthogranuloma	47	47	8.5	Poor
Cappello et al.[83]	2020	CS	Endoscopic	Pituitary tumor, craniopharyngioma, pituitary apoplexy, cyst, other	125	125	3.2	Fair

CSF: Cerebrospinal fluid
N: Number
RC: Retrospective cohort
PC: Prospective cohort
CS: Case series
[a]Excluded from primary analysis due to overlapping population with Ye et al. 2017[40]
[b]Excluded from primary analysis due to overlapping population with Gondim et al. 2015[23]
[c]Excluded from primary analysis due to overlapping population with Fujimoto et al. 2017[49]
[d]Excluded from primary analysis due to overlapping population with Wilson et al. 2018[67]
[e]Excluded from primary analysis due to overlapping population with Zhang et al. 2020[78]
[f] Excluded from primary analysis due to overlapping population with Chen, Sprau et al. 2019[73]
[g] Excluded from primary analysis due to overlapping population with Memel et al. 2019[75]

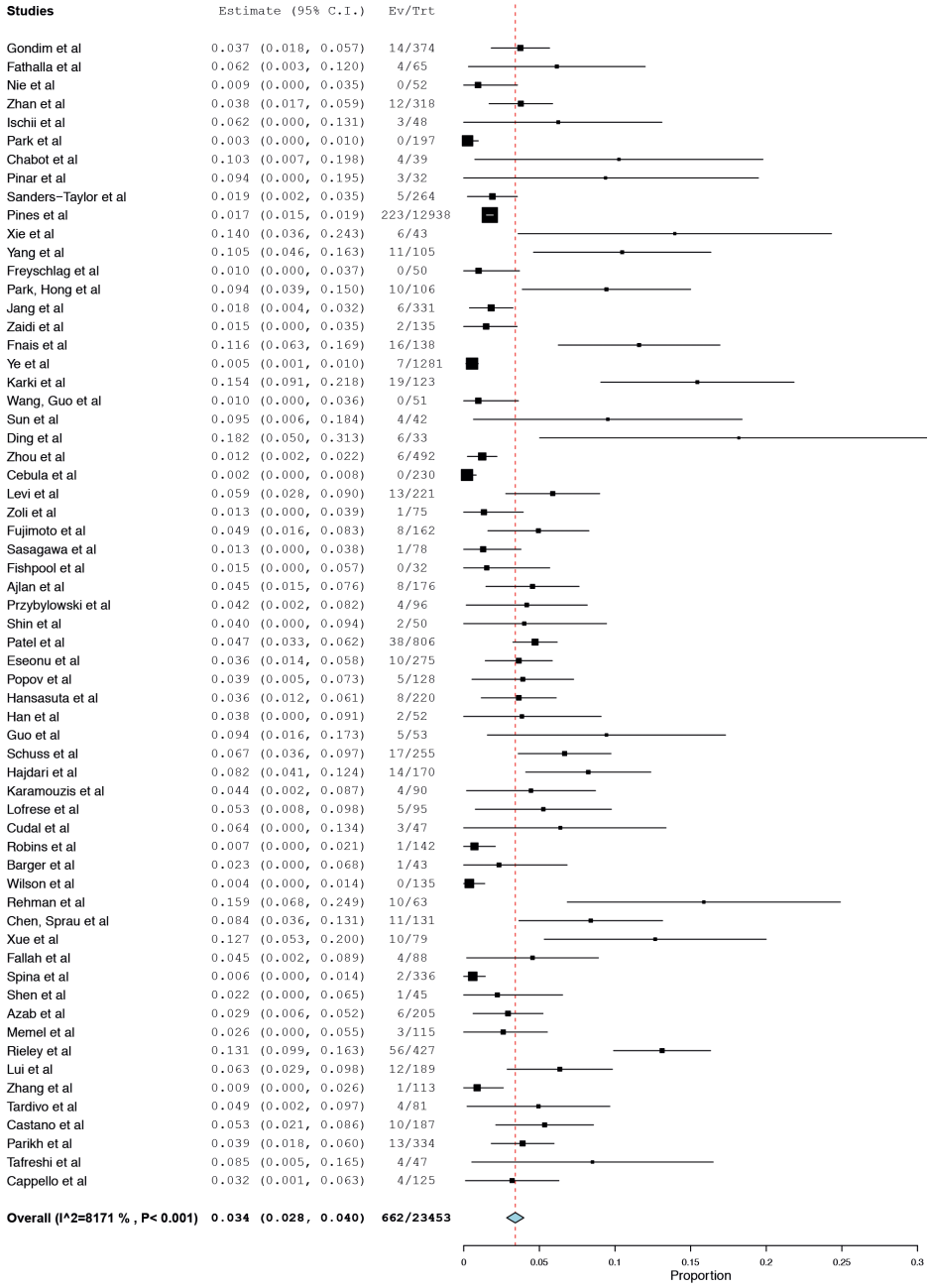

Figure 2. Forest plot prevalence of cerebrospinal fluid leak

The prevalence of CSF leak found in patients undergoing pituitary adenoma resection was 3.2% (95% CI 2.5-4.2%), whereas patients who underwent TSS for another indication (i.e. craniopharyngioma, meningioma, Ratheke's Cleft cyst) had a CSF leak prevalence rate of 7.1% (95% CI 3.0-15.7%) (OR 2.3, 95% CI 0.9-5.7). Data on further specified diagnosis subgroups is too limited to analyse its influence on CSF leak. In this dataset, there is one study reporting on CSF leak on Rathke's cleft cyst separately in which none of the 19 cases had CSF leak[28]. Three small populations of craniopharyngioma's are included in which a total of 6 out 49 patients had CSF leak (12.2%)[27 28, 44]. Two studies specify CSF leak in meningioma cases, of which 1 out of 15 patients had CSF leak (6.7%)[27, 28]. For 2,318 cases the CSF leak rate was not specified per diagnosis subgroup.

Postoperative CSF leak was observed in 5.5% (95% CI 3.3-9.0%) of microscopically approached cases, as opposed to 4.0% (95% CI 3.0-5.2%) in endoscopic cases (OR 1.4, 95% CI 0.9-2.3).

CSF leak was present in 2.0% (95% CI 0.0-4.9%) of patients with previous TSS as compared to 0.4% (95% CI 0-1.0%) in patients without history of TSS (OR 0.9, 95% CI 0.2-4.5).

The prevalence of CSF leak in patients without intraoperative CSF leak was 0.7%, whereas 4.1% (OR 5.9, 95% CI 3.8-9.0) of patients with intraoperative CSF leak had a postoperative CSF leak. The prevalence of CSF leak in patients without cavernous sinus invasion was 0.5% (95% CI 0.0-1.1%), as opposed to 2.2% (95% CI 0.4-4.1) in patients with cavernous sinus invasion (OR 3.0, 95% CI 1.1-8.7) (**Table 2**).

Table 2. Risk factors for postoperative CSF leak.

Outcome	OR	Lower bound	Upper bound	P-value
Pituitary adenoma resection vs. other	2.3	0.9	5.7	0.07
Microscopic vs. endoscopic	1.4	0.9	2.3	0.18
History of TSS vs. no history of TSS	0.9	0.2	4.5	0.87
Intraoperative CSF leak vs. no intraoperative CSF leak	5.9	3.8	9.0	0.00*
Cavernous sinus invasion vs. no cavernous sinus invasion	3.0	1.1	8.7	0.04*

CSF: Cerebrospinal fluid
OR: Odds Ratio
TSS: Transsphenoidal surgery
* Significant

Sensitivity analyses

When the study from Pines et al. (2015)[32] is excluded from the overall analysis, the results are comparable 3.7% (95% CI 3.1-4.4%) to the primary outcome analysis (3.4%, 95% CI 2.8-4.0%).

The sensitivity analysis only including high quality studies also shows comparable results to the primary outcome analysis with a CSF leak rate of 3.6% (95% CI 2.3-4.8%).

Analysis of studies between 2015-2017 shows a CSF leak rate of 2.5% (95%CI 1.9-3.1%). The CSF leak rate is 4.6% (95% CI 3.4-5.8%) in studies published between 2018-2020. This does not provide evidence for a learning curve in studies published between 2015-2020.

Discussion

This meta-analysis shows that postoperative CSF leak occurs in 3.4% of adults undergoing transsphenoidal surgery (TSS). Patients with cavernous sinus invasion are significantly more likely to develop postoperative CSF leak compared to those without cavernous sinus invasion (OR 3.0). Another risk factor for postoperative CSF leak is the presence of an intraoperative CSF leak (OR 5.9).

Historically, TSS is thought to pose high risk of CSF leak. The leak rate found in this study is considerably lower compared to a previous meta-analysis including studies published until 2015[4]. This previous meta-analysis reports a CSF leak rate between 7.5-10.5% for endoscopic endonasal tumor resections (including invasive sinonasal tumors) and 5% for pituitary surgery[4]. A similar trend was observed in another recent meta-analysis CSF leak following extended endoscopic endonasal approach for anterior skull base meningioma[84]. In this study CSF leak decreased from 22% to 4% between 2004 and 2020[84].

The reduced CSF leak rate found in the current study most probably results from a combination of 3 factors. First, improved surgical techniques; approach, sealants, endoscopic visualization and more widely used vascularized nasoseptal mucosal flaps. Second, improved awareness for CSF leak due to initial experiences after more broad indications for (endoscopic) transsphenoidal surgery. Third, improved indication for transsphenoidal surgery. Endoscopic surgery is no longer chosen for part of the larger tuberculum sellae meningioma and craniopharyngioma (with lateral or suprachiasmic extensions) cases in most centers[85, 86].

No evidence for a learning curve is found within the timeframe of the current study (2015-2020). Analysis of subgroups based on publication year to define a learning curve is limited by the variation in inclusion periods of studies published in the same year, the difference in the number of publications from a certain time period reporting CSF leak and that no differentiation can be made on type of pathology based on year of publication which may influence results. Furthermore, publication bias cannot be excluded as a contributing factor to the difference in CSF leak rate observed between the current and previous meta-analyses. Yet, we do not believe this to be the main

factor of influence, considering that publication bias may to some extent have also affected studies in the past. Furthermore, there is wide variance in leak rate reported in included studies and studies of small sample size, most vulnerable for publication bias were excluded.

Moreover, the overall prevalence of postoperative CSF leak after TSS is considerably lower than that reported in meta-analyses for craniotomy (8%) and spinal surgery (14%)[87-88]. However, this does not apply to all indications for TSS. CSF leak after TSS for other indications than pituitary adenoma resection is comparable to that found for cranial surgery, including infratentorial surgery, known to be more vulnerable to CSF leak[87]. The relatively low overall leak rate in this meta-analysis may be a result of the relatively high number of pituitary adenoma's included, which may represent a patient population with few additional risk factors, ameliorating the risk of postoperative CSF leak.

Furthermore, a broad range of leak prevalences (0.0-18.2%) was reported by the included studies, resulting in substantial heterogeneity in the meta-analysis. The variation between studies could be explained by the fact that we have included TSS for various indications, which may differ in presence of patient and surgery related risk factors. This is reflected by the results of our subgroup analyses in which we find a relatively low CSF leak rate of 3.2% for pituitary lesions and a substantially higher prevalence of 7.1% for other indications.

However, CSF leak prevalences vary considerably within different subgroups, for example, including standard extradural pituitary surgery only. This can theoretically be explained by different surgical techniques and closure techniques.

Despite the significant improvement in surgical techniques, cavernous sinus invasion is still a considerable factor in CSF leak due to its need for extensive surgery[89]. This may indicate that tumors infiltrating the cavernous sinus are likely to cross the diaphragm thereby increasing the risk of postoperative CSF leak. As definitions of cavernous sinus invasion may vary, we classified Knosp grades 3 and 4 as invasive for this meta-analysis. This finding also further explains the difference in CSF leak between various surgical indications. As craniopharyngiomas and meningiomas are intradural intra-arachnoid lesions, there will certainly be intraoperative leak and thus higher risk of postoperative CSF leak, compared to extra-arachnoid pathology such as pituitary adenomas.

It was postulated by other authors that reoperation in patients with previous transsphenoidal surgery tends to result in incomplete repair of intraoperative CSF leak, which may result in higher rates of postoperative CSF leak[90]. Although CSF leak was present in 2.0% of patients with previous TSS as opposed to 0.4% in patients who underwent primary TSS, our meta-analysis does not find a significant association

between previous transsphenoidal surgery and postoperative CSF leak (OR 0.9, 95% CI 0.2-4.5). However, this effect may be influenced by the limited number of studies reporting TSS as a potential risk factor.

To our knowledge this meta-analysis includes the largest patient population thus far, including over 25,000 cases. Furthermore, it only includes publications from the last 5 years, thereby providing an up-to-date overview of the current situation with state-of-the-art techniques.

One limitation of this study is that the outcome CSF leak is defined differently across studies, this may further explain the variation in reported leak rates across studies. For example, Zaidi et al.[38] define CSF leak as "CSF leak requiring intervention", for other studies CSF leak was taken into consideration only if confirmed by β2-transferrine testing[66,77]. Furthermore, the majority of included studies do not clearly describe their definition of CSF leak which may have caused differences in postoperative CSF leak percentages. Although, self-limiting CSF rhinorrhea is very rare, not all patients require intervention by reoperation, which may result in lower reporting of CSF leak in studies incorporating the need for surgical repair in their definition[47,57].

Secondly, the results of the current meta-analysis are mostly based on retrospective cohort studies and case series, of which a substantial number is of limited sample size. The outcome of this meta-analysis may be subject to publication bias, contributing to the striking difference in postoperative CSF leak rate found for TSS compared to cranial and spinal surgery, as well as previous meta-analyses on TSS.

Thirdly, some of the analyses are based on a limited number of cases. The analysis comparing endoscopic versus microscopic surgery could be performed for a limited number of studies, showing a higher leak rate for microscopic surgery, yet no significant difference. This result should therefore be interpreted with some caution. We find a substantially higher prevalence of CSF leak for TSS for indications other than pituitary adenoma resection. Again, this result is not statistically significant. Yet, the effect may be underestimated by the relatively low number and small sample size of studies reporting on other indications than pituitary adenoma resection.

Fourthly, no meta-analyses could be performed for a number of potentially important factors due to insufficient data, for example: suprasellar extension, dural invasion, BMI, microadenoma vs. macroadenoma, use of preventative external lumbar drainage or reconstruction technique. We did not exclude studies based on their skull base reconstruction technique, which means that all types of reconstruction were included. Many recent studies have focused on different sellar reconstruction techniques. In the current review no analyses were possible to compare specific techniques as there was

insufficient data from the included studies. Nevertheless, this factor could be a cause of the broad range of leak prevalences. Similarly, factors such as BMI, especially in combination with increased intracranial pressure, and extension of the tumor may have an influence on CSF leak. The effects of these potential influences could not be studied in the current review which limits the generalizability of the overall results.

Lastly, studies with fewer than 30 subjects were excluded from this meta-analysis. Therefore, studies on patients with rare pathology (such as tuberculum sellae meningioma) specifically, may be underrepresented in the current meta-analysis. This may have led to an underestimation of the overall CSF leak incidence after TSS.

The results of this meta-analysis underline that CSF leak after TSS for intradural and invasive lesions, such as craniopharyngiomas or tuberculum sellae meningiomas is a clinically relevant problem. To further improve the advancement of TSS for these indications effective solutions to prevent postoperative CSF leak are warranted. Future research should focus on effective closure techniques including augmented dural repair to prevent intraoperative CSF leak for this type of surgery especially. The outcomes of this meta-analysis could serve as a benchmark for future prospective studies on novel techniques to prevent CSF leak after transsphenoidal surgery.

Conclusion

The overall prevalence of CSF leak after TSS in the adult population is 3.4%. Variables of influence are the presence of intraoperative CSF leak and cavernous sinus invasion.

Declarations

Author Contributions

EMHS, RS, EHJV and TPCvD contributed to the study conception and design. EMSH and RS performed the literature search and data collection. The first draft of the manuscript was written by EMHS and RS. Supervision: TPCvD, EHJV, EWH. All authors critically revised the final manuscript.

Standards of reporting

The authors adhered to the PRISMA guidelines.

Conflicts of interest

TPCvD is a consultant for Polyganics B.V..

Funding
EMHS receives a research grant through Polyganics B.V..

Ethics approval
Not applicable

Informed consent
Not applicable

Consent to publish
Not applicable

Acknowledgements

We thank N.P.A. Zuithoff, PhD for his valuable guidance and contribution to the statistical analyses.

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Appendix A: Pubmed search

("Hypophysectomy"[Mesh] OR Transsphenoid*[Title/Abstract] OR Trans sphenoid*[Title/Abstract] OR Endoscopic endonasal[Title/Abstract])

AND

("Cerebrospinal fluid leak"[Mesh] OR Cerebrospinal fluid leak*[Title/Abstract] OR Cerebro spinal fluid leak*[Title/Abstract] OR Cerebral spinal fluid leak*[Title/Abstract] OR Cerebrospinal fluid rhinorrh*[Title/Abstract] OR Cerebro spinal fluid rhinorrh*[Title/Abstract] OR Cerebral spinal fluid rhinorrh*[Title/Abstract] OR CSF leak*[Title/Abstract] OR CSF rhinorrh*[Title/Abstract])

Appendix B: Embase search

('transsphenoidal surgery'/exp OR 'transsphenoid*':ab,ti OR 'trans sphenoid*':ab,ti OR 'endoscopic endonasal':ab,ti)

AND

('liquorrhea'/exp OR 'cerebrospinal fluid leak*':ab,ti OR 'cerebro spinal fluid leak*':ab,ti OR 'cerebral spinal fluid leak*':ab,ti OR 'cerebrospinal fluid rhinorrh*':ab,ti OR 'cerebro spinal fluid rhinorrh*':ab,ti OR 'cerebral spinal fluid rhinorrh*':ab,ti OR 'csf leak*':ab,ti OR 'csf rhinorrh*':ab,ti)

AND

[embase]/lim

Appendix C: Cochrane library search

MeSH descriptor: [Hypophysectomy] explode all trees

OR

transsphenoid* OR trans sphenoid* OR endoscopic endonasal

AND

MeSH descriptor: [Cerebrospinal Fluid Leak] explode all trees

OR

cerebrospinal fluid leak* OR cerebro spinal fluid leak* OR cerebral spinal fluid leak* OR cerebrospinal fluid rhinorrh* OR cerebro spinal fluid rhinorrh* OR cerebral spinal fluid rhinorrh* OR csf leak* OR csf rhinorrh*

Supplementary Material 1. Overview of quality assessment

Table 1. Overview of quality assessment case series studies.

Study	Question									Quality rating
	Was the study question or objective clearly stated?	Was the study population clearly and fully described, including a case definition?	Were the cases consecutive?	Were the subjects comparable?	Was the intervention (surgery) clearly described?	Were the outcome measures (CSF leakage) clearly defined, valid, reliable, and implemented consistently across all study participants?	Was the length of follow-up adequate (one month or longer)?	Were the statistical methods well-described?	Were the results well-described?	
Park et al[27] (2015)	Yes	Yes	CD	Yes	Yes	No	NR	NA	Yes	**Poor**
Sanders-Taylor et al[30] (2015)	Yes	Yes	Yes	Yes	No	No	NR	No	Yes	**Poor**
Fishpool et al[50] (2017)	Yes	No	Yes	Yes	No	No	Yes	No	Yes	**Poor**
Sun et al[42] (2017)	Yes	Yes	CD	Yes	Yes	No	NR	NA	Yes	**Poor**
Ye et al[39] (2017)	Yes	Yes	CD	Yes	Yes	No	No	No	Yes	**Poor**
Gondim et al[15] (2017)	Yes	Yes	CD	Yes	No	No	Yes	NA	Yes	**Poor**
Cudal et al[63] (2018)	Yes	Yes	Yes	Yes	No	No	NR	NA	Yes	**Poor**
Tafreshi et al[81] (2020)	Yes	Yes	CD	Yes	No	No	NR	Yes	Yes	**Poor**
Nie et al[24] (2015)	Yes	Yes	CD	Yes	Yes	No	Yes	NA	Yes	**Fair**
Pinar et al[29] (2015)	Yes	Yes	CD	Yes	Yes	No	Yes	NA	Yes	**Fair**
Wang et al[14] (2015)	Yes	Yes	Yes	Yes	Yes	No	Yes	NA	Yes	**Fair**
Chabot et al[28] (2015)	Yes	Yes	Yes	Yes	Yes	No	NR	No	Yes	**Fair**
Freyschlag et al[34] (2016)	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	**Fair**
Yano et al[16] (2017)	Yes	Yes	CD	Yes	No	No	Yes	Yes	Yes	**Fair**
Shin et al[53] (2017)	Yes	Yes	CD	Yes	No	No	Yes	Yes	Yes	**Fair**
Zoli et al[47] (2017)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	**Fair**
Karamouzis et al[61] (2018)	Yes	Yes	Yes	Yes	No	No	NR	Yes	Yes	**Fair**
Hansasuta et al[56] (2018)	Yes	Yes	Yes	Yes	Yes	Yes	NR	No	Yes	**Fair**
Hajdari et al[60] (2018)	Yes	Yes	Yes	Yes	Yes	No	NR	Yes	Yes	**Fair**
Rehman et al[67] (2018)	Yes	Yes	Yes	Yes	Yes	No	NR	Yes	Yes	**Fair**
Chen et al[72] (2019)	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	**Fair**
Eichberg et al[19] (2019)	Yes	Yes	Yes	Yes	Yes	No	NR	NA	Yes	**Fair**
Shen et al[71] (2019)	Yes	Yes	CD	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Seltzer et al[20] (2019)	Yes	Yes	Yes	Yes	No	No	Yes	NA	Yes	**Fair**

Table 1. *(continued)*

Study	Question									Quality rating
	Was the study question or objective clearly stated?	Was the study population clearly and fully described, including a case definition?	Were the cases consecutive?	Were the subjects comparable?	Was the intervention (surgery) clearly described??	Were the outcome measures (CSF leakage) clearly defined, valid, reliable, and implemented consistently across all study participants?	Was the length of follow-up adequate (one month or longer)?	Were the statistical methods well-described?	Were the results well-described?	
Pangal et al[21] (2020)	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	**Fair**
Zhang et al[77] (2020)	Yes	Yes	Yes	Yes	Yes	No	NR	NA	Yes	**Fair**
Cappello et al[82] (2020)	Yes	Yes	Yes	Yes	Yes	No	NR	NA	Yes	**Fair**
Jang et al[36] (2016)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Good**
Negm et al[17] (2017)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Good**
Han et al[57] (2018)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Good**
Barger et al[65] (2018)	Yes	Yes	Yes	Yes	Yes	Yes	NR	Yes	Yes	**Good**
Fallah et al[69] (2019	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Good**
Parikh et al[80] (2020)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	**Good**

Legend

CD	NA	NR	Yes	CD/NA/NR	No
Can not determine	Not applicable	Not reported	Low risk of bias	Unclear risk of bias	High risk of bias

Table 2. Overview of quality assessment cohort studies.

Study	Bias								Quality rating
	Representativeness of the Exposed Cohort	Selection of the Non-Exposed Cohort	Ascertainment of Exposure	Demonstration That Outcome of Interest Was Not Present at Start of Study	Comparability of Cohorts on the Basis of the Design or Analysis	Assessment of Outcome	Was Follow-Up Long Enough for Outcomes to Occur	Adequacy of Follow Up of Cohorts	
Ischii et al[26] (2015)	*	*	*	*	-	*	B	D	**Poor**
Pines et al[31] (2015)	*	*	*	*	-	*	B	D	**Poor**
Fujimoto et al[48] (2017)	*	*	*	*	-	*	B	D	**Poor**
Levi et al[46] (2017)	*	*	*	*	-	*	B	D	**Poor**
Schuss et al[59] (2018)	*	*	*	*	-	*	B	D	**Poor**
Robins et al[64] (2018)	*	*	*	*	-	*	B	D	**Poor**
Rieley et al[75] (2020)	*	*	*	*	-	*	B	D	**Poor**
Zhan et al[25] (2015)	*	*	*	*	**	*	B	D	**Fair**
Zaidi et al[37] (2016)	*	*	*	*	**	*	B	D	**Fair**
Gao et al[33] (2016)	*	*	*	*	-	*	B	*	**Fair**
Park et al[35] (2016)	*	*	*	*	-	*	*	D	**Fair**
Ajlan et al[51] (2017)	*	*	*	*	-	*	*	D	**Fair**
Cebula et al[45] (2017)	*	*	*	*	-	*	*	*	**Fair**
Fnais et al[38] (2017)	*	*	*	*	-	*	B	*	**Fair**
Zhou et al[44] (2017)	*	*	*	*	*	*	B	*	**Fair**
Sasagawa et al[49] (2017)	*	*	*	*	**	*	B	D	**Fair**
Patel et al[4] (2018)	*	*	*	*	**	*	B	D	**Fair**
Guo et al[58] (2018)	*	*	*	*	**	*	B	D	**Fair**
Lofrese et al[62] (2018)	*	*	*	*	**	*	B	D	**Fair**
Popov et al[55] (2018)	*	*	*	*	**	*	B	D	**Fair**
Xue et al[68] (2019)	*	*	*	*	**	*	B	D	**Fair**
Memel et al[74] (2019)	*	*	*	*	-	*	*	D	**Fair**
Castano et al[79] (2020)	*	*	*	*	**	*	B	D	**Fair**
Liu et al[76] (2020)	*	*	*	*	*	*	B	D	**Fair**
Fathalla et al[23] (2015)	*	*	*	*	*	*	*	*	**Good**
Gondim et al[22] (2015)	*	*	*	*	**	*	*	*	**Good**
Xie et al[32] (2016)	*	*	*	*	**	*	*	D	**Good**
Przybylowski et al[52] (2017)	*	*	*	*	**	*	*	*	**Good**
Ding et al[43] (2017)	*	*	*	*	*	*	*	*	**Good**

Table 2. *(continued)*

Study	Bias								Quality rating
	Representativeness of the Exposed Cohort	Selection of the Non-Exposed Cohort	Ascertainment of Exposure	Demonstration That Outcome of Interest Was Not Present at Start of Study	Comparability of Cohorts on the Basis of the Design or Analysis	Assessment of Outcome	Was Follow-Up Long Enough for Outcomes to Occur	Adequacy of Follow Up of Cohorts	
Wang et al[41] (2017)	*	*	*	*	**	*	*	D	**Good**
Karki et al[40] (2017)	*	*	*	*	**	*	*	D	**Good**
Wilson et al[66] (2018)	*	*	*	*	**	*	*	D	**Good**
Eseonu et al[54] (2018)	*	*	*	*	**	*	*	D	**Good**
Chen et al[18] (2019)	*	*	*	*	**	*	B	*	**Good**
Spina et al[70] (2019)	*	*	*	*	**	*	*	*	**Good**
Azab et al[73] (2019)	*	*	*	*	**	*	*	D	**Good**
Tardivo et al[78] (2020)	*	*	*	*	**	*	*	D	**Good**

Legend

B	D	*/**	B/D	-
No	No statement	Low risk of bias	Unclear risk of bias	High risk of bias


5

Ex vivo and in vivo evaluation of transsphenoidal Liqoseal application to prevent cerebrospinal fluid leakage

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Acta Neurochirurgica - 2023. doi.org/10.1007/s00701-022-05477-3. Epub ahead of print.

Abstract

Background

Despite improvements in closure techniques by using a vital nasoseptal flap, the use of sealing materials and improved neurosurgical techniques, cerebrospinal fluid (CSF) leak after transsphenoidal surgery still is a clinically relevant problem. Liqosael® (Polyganics bv, Groningen, The Netherlands) is a CE approved bioresorbable sealant patch for use as an adjunct to standard methods of cranial dural closure to prevent CSF leakage. This study aims to evaluate the application of Liqoseal in transsphenoidal surgery ex vivo and in vivo.

Methods

1. We created an ex vivo setup simulating the sphenoidal anatomy, using a fluid pump and porcine dura positioned on a conus with the anatomical dimensions of the sella to evaluate whether the burst pressure of Liqoseal applied to a bulging surface was above physiological intracranial pressure. Burst pressure was measured with a probe connected to dedicated computer software. Because of the challenging transsphenoidal environment we tested in 4 groups with varying compression weight and time for the application of Liqoseal.

2. We subsequently describe the application of Liqosael® in 3 patients during transsphenoidal procedures with intraoperative CSF leakage to prevent postoperative CSF leakage.

Results

1. Ex vivo: The overall mean burst pressure in the transsphenoidal set up was 231 (+- 103) mmHg. There was no significant difference in mean burst pressure between groups based on application weight and time (p=0.227).

2. In Vivo: None of the patients had a postoperative CSF leak. No nose passage problems were observed. One patient had a postoperative meningitis and ventriculitis, most likely related to preoperative extensive CSF leakage. Postoperative imaging did not show any local infection, swelling or other device related adverse effects.

Conclusions

We assess the use of Liqosael® to seal a dural defect during an endoscopic transsphenoidal procedure as to be likely safe and potentially effective.

Running Title

Application of Liqoseal in TSS

Keywords
cerebrospinal fluid leakage, case report, device, transsphenoidal surgery

Abbreviations
ANOVA – analysis of variance
CE - Conformité Européenne
CSF – cerebrospinal fluid
ELD – external lumbar drain
EVD – external ventricular drain
MRI – magnetic resonance imaging
mm – millimeters
cm - centimeters
mmHg – millimeters of mercury
N – number
NSF – nasoseptal flap
PEEP – positive end-expiratory pressure
TSS – transsphenoidal surgery
USA – United States of America

Introduction

Cerebrospinal fluid (CSF) leak is a frequent complication after transsphenoidal surgery (TSS), with an overall prevalence of 3.4%.[1] The prevalence of CSF leak for indications other than pituitary adenomas (i.e. craniopharyngioma, meningioma, Rathke's cleft cysts) is 7.1%, which is similar to that found for craniotomies.[1] CSF leak is associated with various complications such as meningitis, CSF hypotension syndrome and intracerebral hemorrhage causing increased morbidity and mortality.[2, 3] Furthermore, hospital costs for patients with CSF leak after TSS are significantly higher than for patients without.[2, 4]

Despite improvements in closure techniques by using a vital nasoseptal flap (NSF), the use of sealing materials and improved neurosurgical techniques, CSF leak after TSS still is a clinically relevant problem, for intradural and invasive lesions, such as craniopharyngiomas or tuberculum sellae meningiomas, especially. Retrospective analyses of the use of a patch sealant, TachoSil (Takeda Pharmaceuticals, Tokyo, Japan), in TSS show variable postoperative CSF leak results ranging from 0.8-7.8%.[5-7] For liquid sealants, Tisseel (Baxter, Deerfield, USA) and DuraSeal (Integra Lifesciences, Princeton, USA), similar results have been reported in retrospective analyses with postoperative CSF leak ranging from 1-12.5%.[8, 9] Pereira et al.[9] did not find a statistically significant difference in postoperative CSF leak for the use of Tisseel® or DuraSeal®. To further improve the advancement of TSS effective solutions to prevent postoperative CSF leak are warranted.

Liqosael® (Polyganics B.V., Groningen, The Netherlands) is a CE (Conformité Européenne) approved bioresorbable sealant patch for use as an adjunct to standard methods of cranial dural closure. The patch is composed of a white foam layer containing Polyethylene glycol-N-hydroxysuccinimide, the adhesive component, and buffer salt.[10] The blue layer is made of polyurethane and provides the watertight seal (**figure 1, chapter 3 page 44**).[10] The first in human study (ENCASE) has shown that the patch is safe and potentially efficacious for reducing CSF leakage after intracranial surgery.[10, 11]

TSS is regarded as a form of cranial surgery, and thus Liqosael® application is not off-label.[12] However, the surrounding tissue and dimensions in this approach are different compared to a craniotomy. Therefore, this study evaluates the application of Liqosael® in TSS in preclinical (ex vivo) setting and 3 endoscopic transsphenoidal cases.

Methods

Ex vivo

Model

We created an ex-vivo transsphenoidal burst pressure model by adapting an earlier published dural sealing modelwith a conus in the shape of the sella to mimic the application area (**Figure 2A).**[13, 14] The dimensions of the conus (17x7.5 mm) were based on measurements of the pituitary gland and sella turcica on 23 anonymized MRI scans of patients with pituitary adenomas.

Cranial porcine dura was harvested at an abattoir and cut into circles with a 30 mm diameter. A circular gap of 3 mm was punched out in the center. Liqosael® was cut into circles of 15 mm in diameter. The dura was clamped above the open pressure chamber and the Liqosael® applied manually to cover the gap from the outside with a 5 mm overlap.

Liqosael® was compressed by equally and continuously applying a standardized weight on a moist gauze for a specified time period. For cranial application of Liqosael® a compression time of 2 minutes with a compression weight of 1 kg was used, to allow optimal adhesion by the formation of amide bonds between the foam layer of the patch and the dura mater.[13] However, the difficult corridor in TSS could, in practice, result in the prescribed application pressure not being met. Therefore, the acute burst pressure was evaluated with a compression weight 1 kg and 0,25 kg. Furthermore, a shorter compression time would be clinically advantageous. Hence, we compared acute burst pressure for compression times of 2 minutes and 1 minute, respectively.

A fluid pump with a constant flow of 2.0 mL/min of artificial CSF (EcoCyte Bioscience, Germany) was used to increase the pressure in the chamber. The pressure was continuously measured using a blood pressure probe (AD instruments MLT0670 Disposable BP transducer) connected to a computer using LabChart v8.1.14 software (ADInstruments, Australia). Burst pressure was defined as the maximum pressure in millimeter of mercury (mmHg) determined on the continuous measurement in LabChart **(Figure 2B)** at the moment of fluid leakage. The aim of these experimental set-up was to determine if Liqosael® would adhere to the dura with mean burst pressure above the higher end of the physiological intracranial pressure range (> 30 mmHg) on a surface resembling the shape of the sella with varying compression weight and time during application.[15]

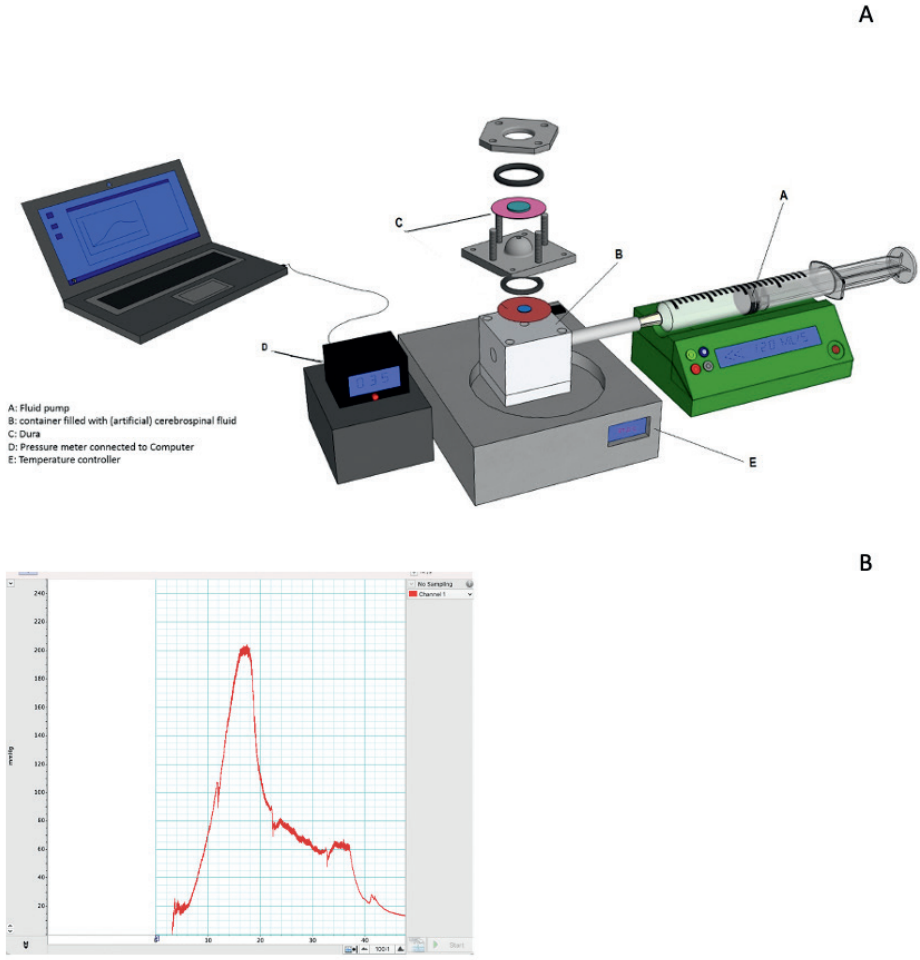

Figure 2. A) Set up for burst pressure measurement. B) Example output of burst pressure software (Labchart, AD Instruments)

Statistics

The required sample size to detect statistically significant differences between groups with an alpha of 0.05 and power of 90% was determined at 23 measurements per subgroup, using the power analysis for One-way Analysis of Variance (ANOVA). Input for sample size calculation was based on the results of the previous cranial and spinal measurements.[13] A total of 3 additional measurements were planned per subgroup to allow for loss of measurements due to experimental failure, so in total 104 measurements were performed. The four groups varying in compression weight (1 kg vs. 0.25 kg) and time (1 min vs. 2 min) were compared using ANOVA. Post hoc Bonferroni correction was applied to adjust for multiple comparisons. Spearman's rank-order correlation was was used to evaluate the association between burst pressure

and interval between measurement and harvesting of the dura. All analyses were performed in SPSS version 27 (IBM).

In vivo

We performed a retrospective evaluation of all transsphenoidal surgeries in which Liqosael® was used in the University Hospital of Zurich, Switzerland, between the 3rd of January 2020 (when Liqosael® was approved) and 1st of March 2022. Three Liqosael® applications were performed in these procedures. Liqosael® was applied on the outside of the defect in all cases. All 3 patients provided a general informed consent for the use of all clinical data and imaging for research.

Results

Ex vivo

A total of 100 measurements were included in the analysis. Four measurements were excluded from the analysis because leakage in the experimental setup prevented adequate pressure built-up. The overall mean burst pressure in the transsphenoidal set up was 231 (± 103) mmHg (**Figure 3, Table 1)**. There was no significant difference in mean burst pressure between groups based on application time and weight (p=0.227).

Spearman's rank-order correlation showed no significant correlation between mean burst pressure and interval between experiment and harvesting (r_s= 0.031, p=0.759).

Table 1. Burst pressure in 4 groups; 1kg/2min, 1kg/1min, 0,25kg/2min, 0,25kg/1min

Group	Mean Burst Pressure (mmHg)	SD	Lowest value	Highest value	N included	N performed
1 kg, 2 min	241,4	135,0	69,4	459,0	25	26
1 kg, 1 min	257,3	102,0	70,1	426,4	24	26
0,25 kg, 2 min	229,5	77,5	53,4	352,6	25	26
0,25 kg, 1 min	199,0	85,1	62,9	397,4	26	26

SD: standard devation
N: number

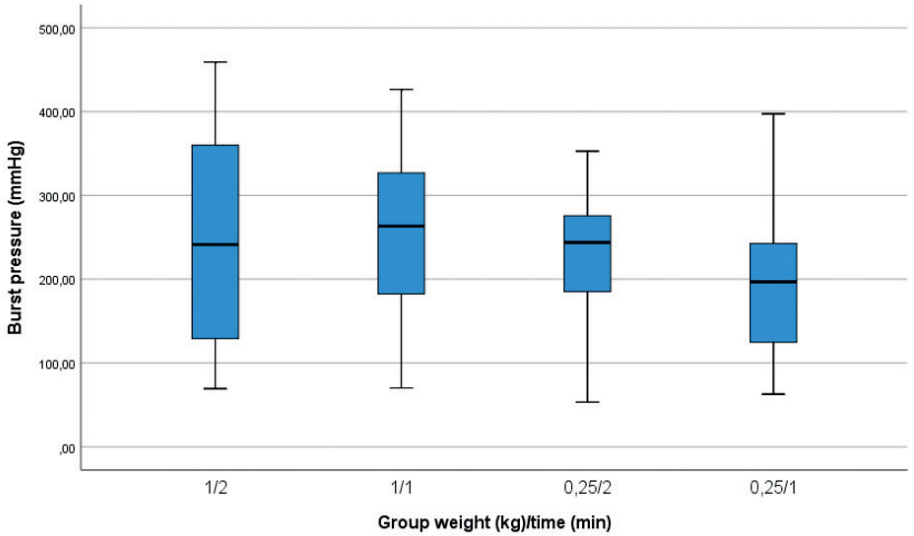

Figure 3. Boxplot (minimum, Q1, median, Q3 and maximum) of burst pressure in 4 groups varying compression weight and time; 1kg/2min, 1kg/1min, 0.25kg/2min, 0.25kg/1min

In Vivo

Liqosael® was applied in 3 endoscopic transsphenoidal surgeries until March 1st, 2022.

Case 1

Patient 1 (63 years old male) was diagnosed with a hormone inactive growing gonadotrophic macroadenoma **(Figure 4, Table 2, Supplementary Information 1)**. Intraoperatively an evident CSF leak occurred **(Figure 5A)**. The patient was operated using the mononostril 'chopstick' approach with the aim to preserve healthy mucosal tissue.[16] Considering the small size of the defect, preparing an NSF resulting in damage to the nasal mucosa was not considered favorable. Therefore, it was decided to seal with Liqosael® combined with external lumbar drainage (ELD). A piece of plastic was used to assess the size of the bony defect in the sella. A circular piece of Liqosael® was cut with 10 mm margin at all sides. After trying several folding options, the piece was folded in 2 with the white side out and parachuted in holding the patch at the front tip to pull the patch forward instead of pushing it. After positioning, a series of small cottonoids was positioned over the Liqosael® before compressing for 2 minutes with a 90-degree ring curette. This led to a good adherence over bone and sella region. However, a small bottom part of the sealant was hampered by loose mucosa. The Liqosael® could be removed with a gentle pulling force via the forceps. The basal bone was cleaned, mucosa removed and a second circular piece of the same patch of Liqosael® was applied that covered the whole sellar defect with a margin of 10mm **(Figure 5B)**. Positive end-expiratory pressure (PEEP) test was performed (20 cm H_2O for 20 seconds) showing no leakage. The

patch was covered with Tisseel® and to prevent the patch from being exposed to air and Spongostan (Ethicon, Raritan, USA) to further cover the patch and mucosa, to fill-up the cavity and provide additional tissue support **(Figure 5C)**. A nasal packing was put in place to further provide support to the surrounding tissues and to tamponade any small bleeding afterwards. Postoperatively, no rhinoliquorrhea was observed. The ELD was removed at day 6. Patient was discharged day 8 after surgery without complications. Three-month endoscopic control showed complete re-endothelialization **(Figure 5D)**. At further MRI follow-up **(Figure 6)** individual patch recognition was not possible, but no signs of infection or swelling of the patch were observed. During the entire follow-up period of 15 months there were no nasal complaints and good olfactory function.

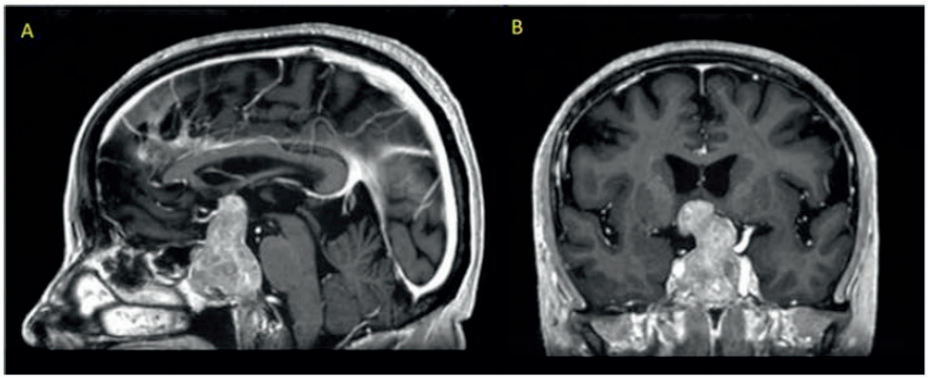

Figure 4. Preoperative MRI patient 1 showing a macroadenoma in A) saggital view and B) coronal view.

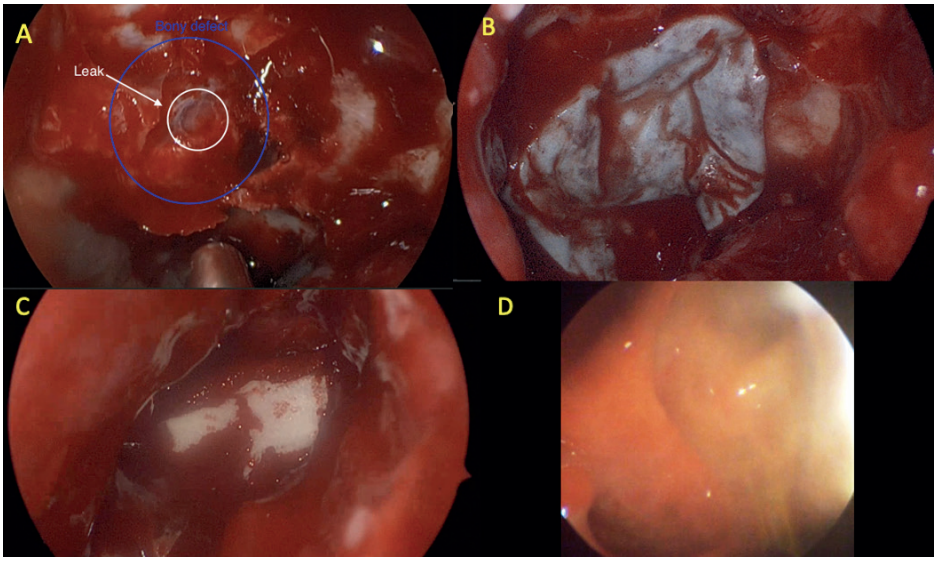

Figure 5. Endoscopic images patient 1 showing A) intraoperative CSF leakage B) final Liqoseal positioning C) intraoperative end situation and D) 3-month follow-up with full re-endothelisation in patient 1

Table 2. Overview of cases

	Age	Sex	BMI	Smoking	Relevant medical history	Indication	Intraoperative Complications	Other closure techniques	External CSF drainage	Postoperative Complications	Discharge (d)	Postoperative treatment	Neurological deficit	Final follow-up (m)
1	63	M	26	Former (20 PY)	None	Macro adenoma	CSF leakage	Tisseel, Spongostan, Fat, Nose tampon	ELD day 0-6	None	7	None	None	15
2	54	F	23	No	2 times TSS	Revision CSF leakage	None	Tisseel, Spongostan, Fat, Nose tampon	ELD day 0-4 EVD day 4-40 VPS at day 40	Meningitis and ventriculitis, Hydrocephalus	44	None	Bitemporal hemianopsia	6
3	7	F	15	No	None	Clival chordoma	CSF leakage	Tisseel, Spongostan, Fat	ELD day 0-8	None	12	Proton beam therapy	Abducens nerve palsy	7

d – days
m – months
BMI – body mass index
CSF – Cerebrospinal fluid
ELD – eternal lumbar drain
EVD – external ventricular drain
VPS – ventriculoperitoneal shunt

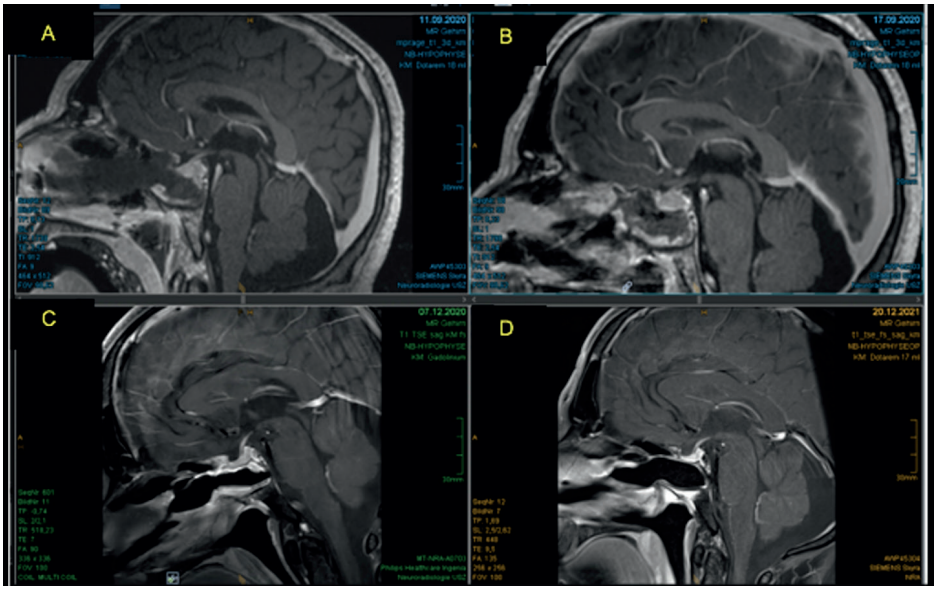

Figure 6. MRI follow-up patient 1 showing smoothening of the sellar wall over time. No signs of infection, swelling or other pathological reactions was observed. A) intraoperative MRI (no Liqoseal) B) day 6 postoperatively C) 3 months postoperatively D) 15 months postoperatively

Case 2

Patient 2 (54 years old female) was diagnosed with a giant macroadenoma causing bitemporal hemianopsia **(Figure 7, Table 2, Supplementary Information 1)**. First surgery (day -17) was complicated by postoperative rhinorliquorrhea. A revision surgery was performed using a vascularized NSF to seal the defect and decreasing CSF pressure with ELD (day -7). The leakage continued postoperatively despite increasing CSF drainage volume. During the second revision surgery (day 0) a defect just above the vital NSF was observed **(Figure 8A)**. As salvage treatment a fat plug was placed in the small defect. Subsequently Liqoseal was inserted with the same method as described in patient 1 **(Figure 8B-C)**. PEEP test (20 cm H_2O for 20 seconds) showed no intraoperative leakage. The patch was covered with Tisseel® and Spongostan®. A nasal packing was put in place. No rhinoliquorrhea was observed after this surgery. Patient developed a combined meningitis and ventriculitis at day 4 after the 3rd surgery, which was treated with intravenous antibiotics. The ELD was exchanged for an external ventricular drain (EVD) at this day to treat the infection and resulting hydrocephalus. The treating neurosurgeon did not consider Liqosael® as the source of the infection, hence the nose was not surgically revised. At day 12 an MRI was made **(Figure 9)**. Individual Liqosael® patch recognition was not possible and there were no signs of infection or swelling of the patch. Temporary closure of the EVD resulted repeatedly in hydrocephalus (still without leakage). Therefore, a ventriculoperitoneal shunt was placed at day 40.

Patient was discharged at day 44. Final follow-up was 6 months after the surgery in which Liqosael® was applied. Visual disturbances persisted. Patient reported no nasal complaints and good olfactory function. She refused further follow-up.

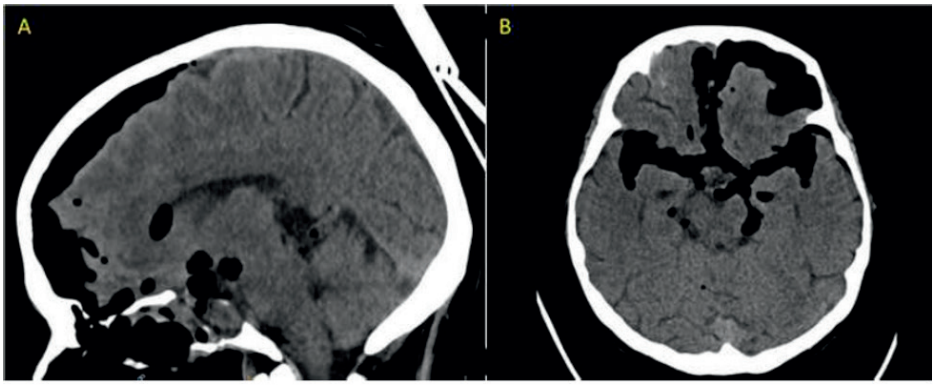

Figure 7. Preoperative CT patient 2 showing pneumocephalus due to CSF leakage after previous surgery in A) sagittal view and B) axial view

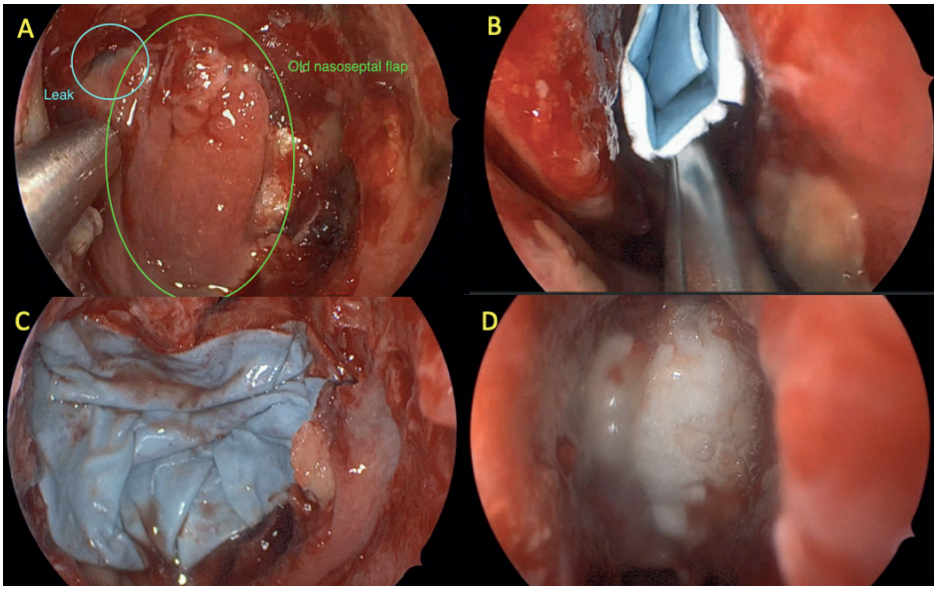

Figure 8. Endoscopic images patient 2 showing A) intraoperative CSF leakage B) folding of Liqoseal during application C) final Liqoseal positioning D) intraoperative end situation in patient 2

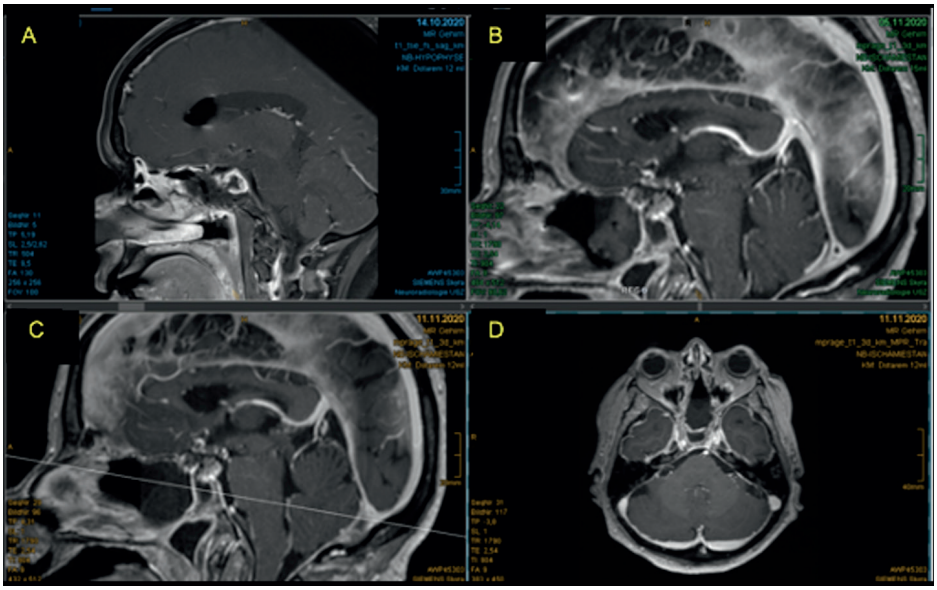

Figure 9. MRI follow-up patient 2 A) 16 days before 3rd surgery B) day 6 after 3rd surgery. C) day 12 postoperatively (sagittal). D) day 12 postoperatively (transversal) showing no swelling of the patch or signs of infection

Case 3

Patiënt 3 (7 years old female) presented with an abducens nerve palsy caused by a clivus chordoma **(Figure 10, Table 2, Supplementary Information 1)**. After resection a large defect in the clivus resulted with a central dural defect **(Figure 11A)**. A NSF was not prepared and it was considered by the operating surgeon that it would be difficult in this case to make it large enough to cover the total defect appropriately. However, no dural sealants have been CE approved for use in children. So on the discretion of the operating surgeons Liqosael® was chosen to be used off-label. This application area was deeper and flatter than in the previous 2 patients. This caused the Liqosael® application to be more difficult and a re-application was necessary. The final positioning showed wrinkles and internal Liqosael® folds **(Figure 11B)**. The operating surgeon however decided to leave the patch in place because the dural defect was covered. The Liqosael® was covered with a fat plug harvested from the periumbilical region **(Figure 11C)**. Tisseel® and fat were thereafter alternately applied. Finally, the construct was covered with Spongostan® to further fill the cavity and deliver additional tissue support (Figure 11D). No PEEP test was performed. Because of the high risk of postoperative leakage associated with the dural defect an ELD was placed intraoperatively as well. Postoperatively, no rhinoliquorrhea was observed. The ELD was removed at day 8. No postoperative complications occurred and patient was discharged at day 12 after surgery. Intraoperative and postoperative MRI showed a small chordoma rest at the

cavernous sinus which was considered inoperable. The patient was radiated with proton beam 7 weeks after surgery. Latest follow-up was at 7 months after surgery. The abducens paresis persisted. Patient showed good nasal passage and olfactory function up until this time. MRI control at this timepoint showed no swelling of the Liqosael® patch and slow resolving of the fat plug **(Figure 12)**.

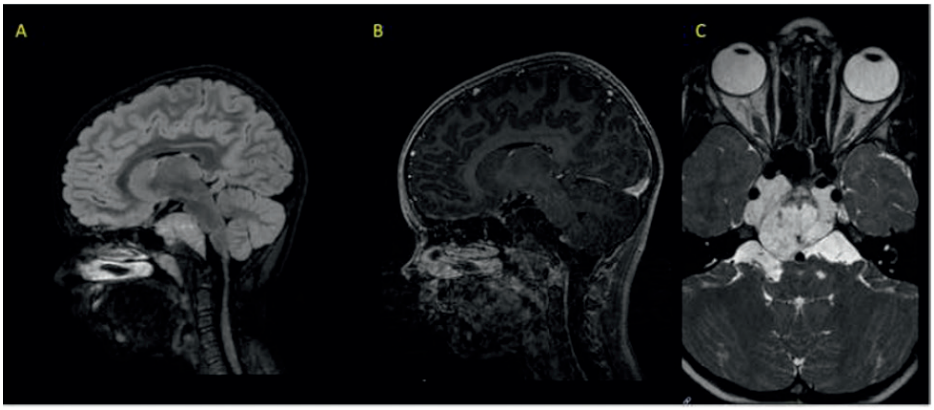

Figure 10. Preoperative MRI showing a clivus chordoma in A and B) sagittal view and C) axial view

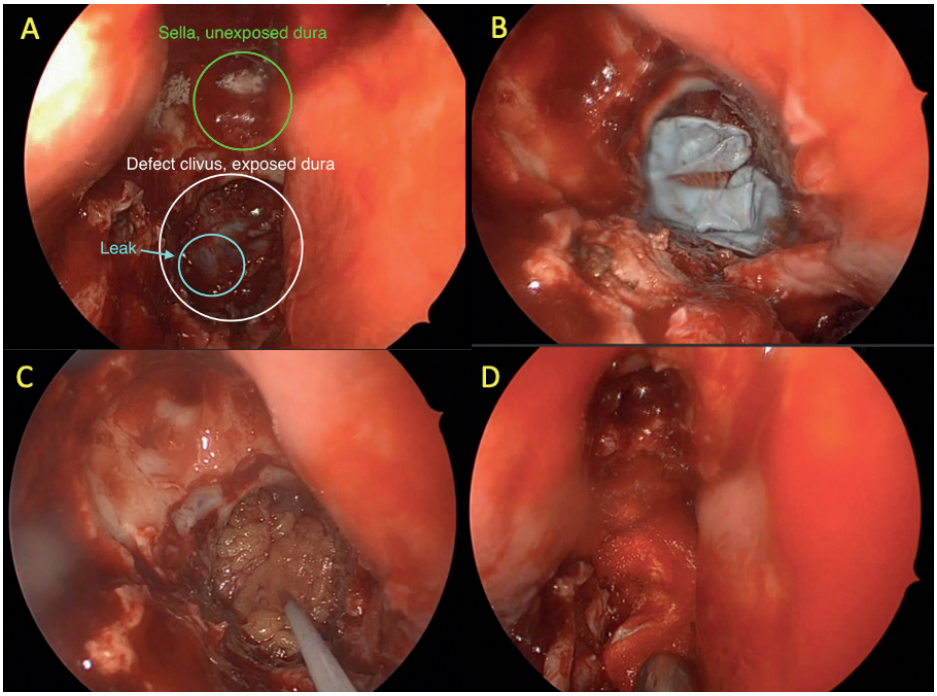

Figure 11. Endoscopic images showing patient 3 A) intraoperative CSF leakage B) final Liqoseal positioning C) fat plug fixated with Tisseel on top of Liqoseal D) intraoperative end situation in patient 3

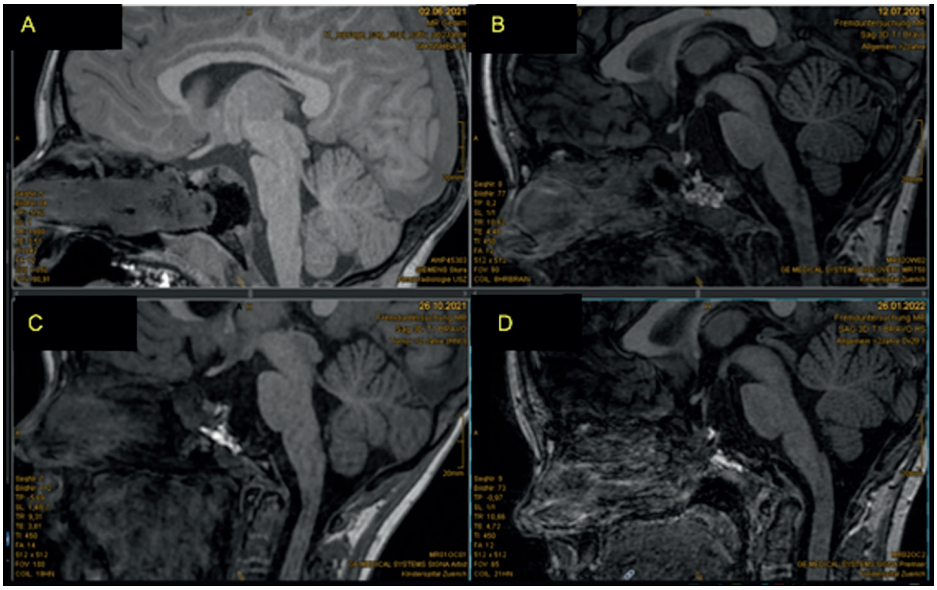

Figure 12. MRI result in patient 3 A) intraoperatively B) 1 month postoperatively C) 5 months post-operatively D) 8 months postoperatively, showing no swelling of the patch or signs of infection. Slow resolving of fat patch can be observed

Discussion

This is the first study that evaluates the application of Liqosael® during TSS. We report excellent ex vivo and in vivo results. The overall mean burst pressure of Liqosael® in this transsphenoidal model (231 ± 103 mmHg) and mean burst pressures in individual groups based on compression weight and time were all well above physiological intracranial pressure.[15] Mean burst pressure in this model was shown to be similar to those found in our cranial and spinal model (145 ± 39 mmHg and 233 ± 81 mmHg, respectively).[13]

Liqosael® was successfully applied during endoscopic endonasal surgery in 3 patients. Given their clinical history each of these patients can be considered as high risk for postoperative CSF leakage. None of these patients postoperative CSF leakage, required revision surgery or had nasal passage problems. There was one infectious complication in patient 2 that occurred 4 days after implantation of the device. This patient was at increased risk for infection because of continuous CSF leakage prior to the surgery in which Liqosael® was applied, and the infection was treatable with antibiotics.[2] We deem the infection unlikely to be device related. We found no indications of safety issues for the transsphenoidal application of Liqosael® based on these 3 patients.

Limitations

The most important limitation of the current study is the small number of TSS cases in which Liqosael® has been applied which does not allow for any conclusions about efficacy. Moreover, all patients in this study received an intraoperative ELD to decrease the CSF pressure and support healing of the dura which may have positively contributed to the prevention of CSF leakage and the functioning of the patch. In addition, fibrin glue (Tisseel®) and gelatin sponge (Spongostan®) was used as a coverage. Furthermore, endoscopic inspection of the nasal mucosa (not standard of care) was performed in one patient only, showing re-endothelization.

Finally, the experimental model was designed based on the sella region. This is representative for the majority of transsphenoidal cases, but not all of them. For example, patient 3 had a clivus tumor that grew under the sella and the surface of this region does not resemble the surface of the ex vivo model. Furthermore, the gap size in the dura in the experimental set up was 3 mm in diameter. In clinical practice the gap size in the dura, especially in cases leading to CSF leak postoperatively, may in fact be larger.

Recommendations

Based on our experience in these first 3 cases, we think that there are a number of technical aspects to take into consideration when applying Liqosael® in TSS. Firstly, we recommend patch sizing to allow for margins of minimally 5 mm, taking into consideration that a larger sized patch is more difficult to introduce. When fat tissue is placed under Liqosael®, we recommend a margin of 10 mm as Liqosael® does not adhere to fat. Secondly, we recommend to fold the patch with the white side (PEG-NHS side) outwards. This has the advantage of easier unfolding, yet does expose the foam layer to possible absorption of blood and damage. Thirdly, the patch should be held at the most distal point with a small rongeur while being introduced in the nose to exert a pulling force on the patch instead of a pushing force. Fourthly, in these 3 cases compression for 2 minutes using moistened cottonoids and a patty was performed with a 90-degree bended ring curette. Despite the results of the ex vivo experiments showing that 1 minute compression appears to be sufficient, we still recommend to compress for a minimum of 2 minutes as stated in the instructions for use for security and consistency reasons. Finally, the dural defects in the cases presented in this article were relatively small. Liqosael® is intended for use on defects with a maximum size of 3 mm. Use over larger defects is thus off-label. We recommend to use Liqosael® in cases with larger defects with caution and only in combination with a construct allowing endothelization and formation of new dura (i.e. covering the mucosal tissue with muscle tissue or fat). It is important to note that Liqoseal does not adhere to fat tissue and that fat tissue will resorb over time. Considering the relatively fast endothelization we have

observed, the primary goal of using Liqosael® in such case is to overcome the time until endothelization without CSF leakage.

Conclusion

The results of this study combined with the outcomes of the ENCASE trial[10, 11] and previous preclinical studies with regard to CSF leakage[1, 13, 14, 17-19] indicate that the use of Liqosael® in the sphenoid sinus to seal a dural defect in TSS is likely safe and potentially effective.

Compliance with ethical standards

Conflict of interest

E.M.H.S received a research grant through Polyganics b.v.

T.P.C.vD is consultant for Polyganics b.v.

Ethics approval

All patients provided written informed consent on the use of their data, including video and photo material, for research and reporting purposes.

Informed consent

General informed consent for the use of all clinical data and imaging for research.

Author contributions

Conceptualization: Tristan van Doormaal; Methodology: Tristan van Doormaal, Nadia Colmer, Emma Slot, Formal analysis and investigation: Emma Slot, Tristan van Doormaal; Surgeries: Tristan van Doormaal, Carlo Serra, David Holzmann, Luca Regli; Writing - original draft preparation: Emma Slot, Tristan van Doormaal; Writing - review and editing: all authors; Supervision: Tristan van Doormaal.

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Supplementary Information 1.
Additional data presentation with regard to the cases.

Supplementary Table 1. Sealing specific variables

Case Nr.	Liqoseal	PEEP test performed	Other sealants	Other closure techniques	Sealing re-application	Long term nose problems/ sealing complications
1	DS01-024/08 Max 2021-08-19 Dur202002511	Yes (20 cm H_2O)	Tisseel, Spongostan	Nasal packing	1 re-application	None (15 months)
2	DS01-024/08 Max: not noted Dur2020091111	Yes (20 cm H_2O)	Tisseel, Spongostan	Fat, nasal packing	None	None (6 months)
3	DS01-024/08 Max 2023-02-12 Dur2020021111	No	Tisseel, Spongostan	Fat	1 re-application	None (7 months)

Nr. = number
PEEP = positive end-expiratory pressure

Supplementary Table 2. MRI information

Case Nr.	MRI 1	MRI 2	MRI max
1	Intraoperative	Day 6	15 months
2	Day 6		Day 12
3	Intraoperative	4 Months	7 months and 3 weeks

MRI: magnetic resonance imaging
Nr. = number

Supplementary Table 3. Body Temperature (°Celsius)

Case Nr.	Day 0 (= surgery)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1.	36.7	37.1	37.3	37.1	36.8	37.2	37.4	37.1
2.	36.8	37.1	37.3	-	39.6	-	-	-
3.	37.2	37.3	37.5	-	37.4	-	-	36.5

Nr. = number

Supplementary Table 4. C-reactive Protein (mg/L)

Case Nr.	Day 0 (= surgery)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 30	Day 90	Max FU
1	0.5	27	-	10	-	-	-	-	-	-	-
2		37	19	18	181	345	222	-	3	-	-
3		9	-	7	-	-	-	-	-	-	-

FU= follow-up
Nr. = number

III

Safety and efficacy of Dura Sealant Patch in transsphenoidal surgery


6

Spinal fixation after laminectomy in pigs prevents postoperative spinal cord injury

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Animal Models and Experimental Medicine. 2022;5(2):153-160. doi: 10.1002/ame2.

Abstract

Background

A safe, effective and ethically sound animal model is essential for preclinical research to investigate spinal medical devices. We report the initial failure of a porcine spinal survival model and a potential solution by fixating the spine.

Methods

Eleven female Dutch Landrace pigs underwent a spinal lumbar interlaminar decompression with durotomy and were randomized for implantation of a medical device or control group. MRI was performed before termination.

Results

Neurological deficits were observed in 6 out of the first 8 animals. Three of these animals were terminated prematurely because they reached the predefined humane endpoint.

Spinal cord compression and myelopathy was observed on post-operative MRI imaging. We hypothesized post-operative spinal instability with epidural hematoma, inherent to the biology of the model, and subsequent spinal cord injury as a potential cause. In the subsequent 3 animals we fixated the spine with lubra plates. All these animals recovered without neurological deficits. The extent of spinal cord compression on MRI was variable across animals and did not seem to correspond well with neurological outcome.

Conclusion

This study shows that in a porcine in vivo model of interlaminar decompression and durotomy, fixation of the spine after lumbar interlaminar decompression is feasible and may improve neurological outcomes. Additional research is necessary to evaluate this hypothesis.

Key words

interlaminar decompression, fixation, spinal cord injury, medical device model

Introduction

A validated and ethically sound animal model is essential for preclinical research to investigate safety and efficacy of biotechnological solutions, such as a sealant to prevent CSF leakage after spinal surgery.

Although there are differences in the loads applied to quadrupedal spine and human spine, various animal models have successfully been used in spine research[1,2]. Most common are; dog, goat, sheep, and pig models[3-5]. Depending on the aim of the study one species may be more suitable than another[5]. Along with factors such as housing and costs, it is important to recognize anatomical differences in the parameters of interest between species in deciding which animal model is best suited for a specific study. For sheep for instance, similarity to the human spine in gross anatomy is greatest for the thoracic and lumbar spine, whereas the trend in vertebral body height is markedly different compared to humans, as this is greatest in the cervical spine of sheep[6,7]. The majority of surgical models in the current body of literature focus on spinal fusion.

Canine models are common for spinal fusion or laminectomy studies, of the cervical as well as lumbar spine[8-12]. On the other hand, dogs that are kept as companion animals frequently undergo spinal surgeries including laminectomies and spinal fixation for spinal disorders similar to humans[13]. Since dogs are companions to humans the use of dogs as experimental animals is therefore less and less accepted, which complicates the use of this species[4].

Goat models have been extensively studied for anterior cervical discectomy and fusion[14]. Lumbar spinal studies in goats are less common[4]. Lumbar spine surgery including instrumentation is frequently performed in sheep models. Porcine models are also often used in lumbar spine surgery, in particular for minimally invasive techniques[15-18]. Both the ovine and porcine model have been used in intradural spinal implant studies as wel[19-21]. It is argued that the porcine animal model is best suited for lumbar spinal research, including implantation, spinal fusion and instrumentation studies, because the porcine spine closely resembles the human spine, especially for the thoracic and lumbar segments[2, 5, 6]. This does however, not take into account that mature pigs are more difficult to handle than some other species because of their size and specific husbandry[4].

Other studies have shown that the porcine spine is a representative model for the human spine and it is often used for training of surgical techniques and preclinical testing[2, 22]. However, this mostly involves instrumentation techniques (i.e. titanium low contact dynamic compression plate for anterior fusion) and minimally invasive surgery[23]. Although a porcine model is preferrable for dural research[24], an *in vivo* porcine model for interlaminar decompression with durotomy has not been extensively researched.

The aim of this study is to share our learning points in an *in vivo* porcine model for interlaminar decompression that was originally designed for a medical device test study.

Methods

The medical device test study was approved by the animal experiment committee (DEC) Utrecht, the Utrecht Animal Welfare Body (IVD) and the Central Animal Experiments Committee affiliated to the Dutch National Institute for Public Health and the Environment (Approval No. AVD1150020184784). The definition of the humane endpoint is reported in Supplementary Material I. The authors followed the ARRIVE guidelines.

Original study design

Eleven female Dutch Landrace pigs with a mean (±SD) body weight of 78.3 (±4.5) kg underwent interlaminar decompression, followed by durotomy and closure of the dura with sutures. Mean body weight at the end of the study was 78.4 (±3.0) kg. No inclusion or exclusion criteria were applied. The pigs were randomized into two groups and in addition to sutures the experimental group (n=8) received a dura sealant patch (DSP), whereas the control group (n=3) did not. Randomization was performed using sealed envelopes. We did not control for confounders. The animals were housed in groups in a dedicated animal laboratory facility and were acclimatized for at least 7 days preoperatively. Two neurosurgeons (TPCvD and BdB) performed the surgeries between November 2018 and May 2019. The surgeons were blinded to group allocation until directly after dural closure.

Anesthesia and surgical procedure

The surgical procedure and MRI were performed under general anesthesia. Intravenous midazolam (0.2 mg/kg) and ketamine (10 mg/kg) were used as premedication, after which anesthesia was induced with thiopental (3-8 mg/kg) and atropine (0.05 mg/kg). Propofol (4.5 mg/h) and remifentanil (0.0066 mg/h) intravenously were used for continuous sedation.

The animal was positioned in ventral recumbency. A routine approach was made to the dorsal thoracolumbar spine. Pigs have a variable number of thoracic vertebrae. Therefore, the upper level was defined as the last thoracic vertebrae-L1[25]. Interlaminar decompression (ILD) was performed at the lumbar spine **(Figure 1A and 1B Supplementary Material II)**. An interlaminar opening of 2 (length) x1 (width) centimeter (cm) (minimum) was made by partially removing the spinous processes, laminae and the ligamentum flavum. A durotomy of 1.5 cm was made on all operated levels throughout the study. The dura was sutured with coated polyglactin 910 (Vicryl 5.0 RB 1 Plus, Ethicon, Somerville, USA) at all operated levels in all control and

experimental animals. The DSP (length 2 cm x width 1 cm) was applied on all operated levels in all experimental animals. **Figure 1** shows an image of the surgical model.

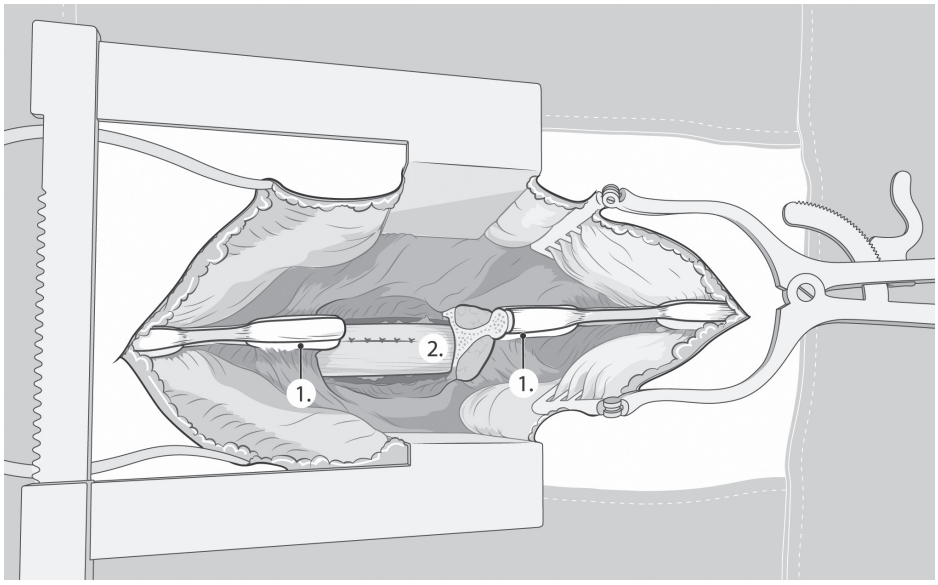

Figure 1. Intra-operative dorsal view of ILD at one level (L1-2), showing sutured durotomy. 1= spinous process, 2=dura mater. Scientific illustration by Amanda Gautier.

ILD and durotomy was performed at 3 levels for pigs # 1-4 (last thoracic vertebrae-L1, L1-2, L3-4, L5-6, L5/6-S1). Due to advancing insights based on the postoperative outcome the procedure was adapted for animals 5 to 11. The number of levels of ILD and durotomy was reduced to 2 levels for pigs # 5-6 (L3-4, L5-6 and L2-3, L4-5, respectively) and 1 level for pigs # 7-11 (L4-5, L5-6, L1-2, L2-3, L3-4, respectively).

The DSP was applied on all operated levels in experimental animals: pig # 1, 2 and 4 (9 levels), pig # 6 (2 levels), pig # 7 and 8 (2 levels), and pig # 9 and 11 (2 levels) (**Figure 1C-D, Supplementary Material II** show the surgical site before and after application of DSP). Pigs # 3 (3 levels) # 5 (2 levels) and pig # 10 (1 level) served as control animals and did not receive a DSP.

Further adaptations were made for animals 7 to 11. In pigs # 7 to 11 a low vacuum wound drain (wound drainage system 40 ml CH6, Medinorm GmbH, Spiesen-Elversberg, Germany) was introduced following ILD, durotomy and application of the DSP. The wound drain was kept in place for at least 1 day until it was no longer productive, with a maximum of three days postoperatively. In pigs # 9 to 11 the spine was stabilized using two 14.5 cm Lubra plates (Veterinary Orthopedic Implants Inc, St. Augustine, USA) and

complementary screws 0.75 inch (Veterinary Orthopedic Implants Inc, St. Augustine, USA). **Figure 2** shows an intraoperative view of the application of the Lubra plates. All products used during the procedure are reported in Supplementary material II **(table 1)**.

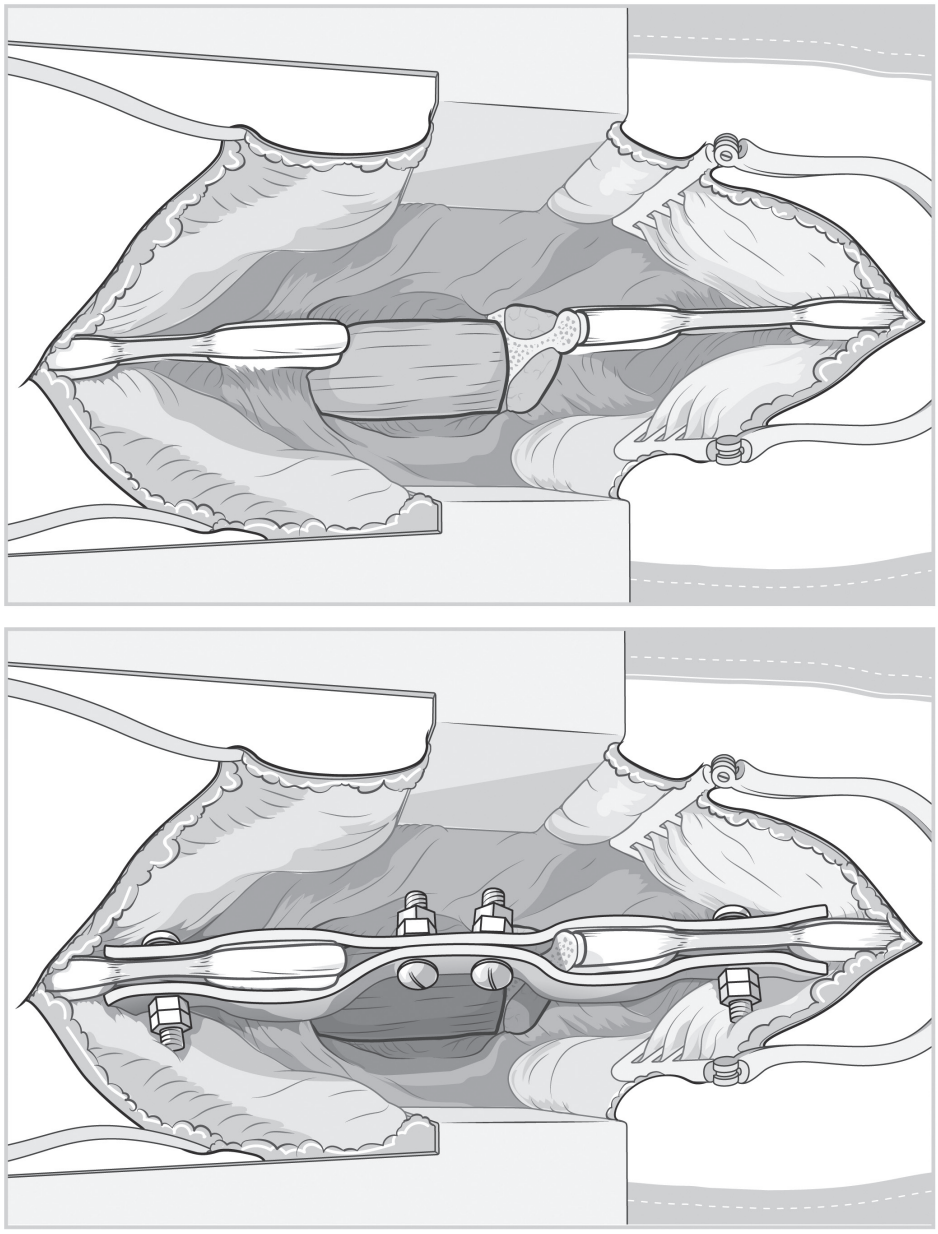

Figure 2. Intraoperative dorsal view of two Lubra plates applied to the lumbar spine after ILD. 1= spinous process, 3=lubra plate, 4=screw. Scientific illustration by Amanda Gautier.

Postoperative procedures

After surgery the pigs were housed solitary in stables. Clinical observations were performed daily by trained laboratory animal care takers in consultation with the veterinarian. Observations were noted on a standardized scoring list (Supplementary Material III). This scoring list was created to collect the outcomes for the medical device test study for which this model was originally used. If neurological deficit was observed an explanation of the observed deficit was reported.

Postoperatively, the pigs received 1 dose of 0.4 mg/kg meloxicam (Metacam, Boehringer Ingelheim, Ingelheim am Rhein, Germany) intramuscularly or intravenously for the first 3 days. Oral antibiotic treatment amoxycillin/clavulanic acid (Synulox, Pfizer Animal Health, Louvain-la-Neuve, Belgium) 1 dose of 10mg/kg daily as well as local antibiotic treatment with neomycine/procaine benzylpencillin (Neopen, Intervet Productions, Aprilia, Italy): 100mg/ml at the intravenous access and surgical wound was administered for 7 days.

Magnetic resonance imaging

Before termination, MRI was performed under anesthesia using a Philips Ingenia 1.5T MRI scanner (Philips, Eindhoven, The Netherlands). The pigs were positioned in sternal recumbency and the following sequences of the thoracal and lumbar spinal axis were performed: T1-weighted (T1W), T2W, flair, and T1W with contrast. A board-certified veterinary radiologist (IS), blinded for group allocation, evaluated the MRI for signs of spinal cord compression (SCC) and myelopathy.

The dorsal to ventral diameter of the normal spinal cord closest to the cranial section of maximum compression was measured on transverse T2W images. Also, the dorsal to ventral diameter of the spinal cord at the level of maximum compression was measured. The degree of SCC was calculated as (normal spinal cord dorsal to ventral diameter, minus spinal cord diameter at maximum compression) divided by (normal spinal cord diameter) multiplied by 100%[26]. The severity of compression was defined as follows; no compression: 0%, mild: <25%, moderate: 25-50% and severe: >50%[27]. At the level of the conus medullaris the measurement was not performed because the natural anatomical diameter of the spinal cord decreased at this location.

Indication of spinal cord myelopathy was evaluated by measuring the trajectory length of hyperintensity of the spinal cord parenchyma (lesion(s)) on sagittal T2W images in millimeter (mm) at each operated level. The length of the vertebral body L5 was measured from cranial endplate to caudal endplate in mm. The extent of the lesion(s) was defined as the ratio between the length of the lesion(s) (hyperintensity on sagittal T2W images) and the length of the vertebral body L5[28].

Directly after MRI, the animals were euthanized with an overdose of pentobarbital 220mg/kg (Euthanimal 40%, Alfasan, Woerden, Netherlands) at day 7 (±1) postoperatively. A humane endpoint allowed for earlier termination.

Results

Clinical outcome

Six out of 11 animals had neurological deficits postoperatively **(Table 1)**. In the animals operated on 3 levels (#1-4), 3 out of 4 had neurological deficits postoperatively. Pig #1 had complete paralysis of the hind legs and pig #2 and #4 severe paresis of the hind legs. These animals were terminated before the study end because the humane endpoint was reached. Pig #3 recovered without postoperative complications.

The subsequent 2 animals (#5-6) were operated on two levels. Pig #5 and #6 suffered from paresis of the hind legs with ability to stand and walk with support. In animal #7 and #8 ILD was performed on one level and a wound drain was added to the surgical protocol to reduce compression of the spinal cord by postoperative oedema and wound fluid. Delayed paresis of the hind legs was present in pig #7 at day 6. Pig #8 recovered well and returned to normal ambulation.

The final 3 animals (#9-11) were operated on 1 level with a wound drain and fixation of the spine by Lubra plates. These animals all recovered well and returned to normal ambulation. Blood analysis and figures of the surgical wounds are presented in Supplementary Material II (**Table 2 and figure 2**, respectively).

MRI

Severe SCC was present in two animals (#8, 9) **(Figure 3)**. Moderate compression of the spinal cord was present in 7 animals (#1-3, 5-7, 10) **(Figure 3, Table 1)**. The remaining 2 animals (#4, 11) had mild compression **(Table 1)**. Evidence of myelopathy (hyperintensity of the spinal cord parenchyma on T2) was seen on MRI in all animals **(Table 1)**. The mean lesion-length-to-vertebral-length ratio was 1.9 (range 0.7-3.3). The 4 highest lesion-length-to-vertebral-length ratios were found in the 4 out of 6 animals with neurological deficits **(Table 1)**. The Lubra plates and fixation material dorsal to the spinal canal allowed sufficient visualization of the spinal cord and dura mater on MRI. The figures of the MRIs of all animals are included in Supplementary Material IV **(figure 1-4)**.

Table 1. Overview of study design and clinical outcome in 11 pigs that underwent lumbar interlaminar decompression (ILD), durotomy and dura sutures.

Pig #	Levels ILD	DSP	Wound drain	Fixation	Neurological deficits	Termination (days)	Maximal degree of SCC on MRI	Maximal SCC on MRI (%)	Lesion length (mm)	Lesion length to vertebral length ratio
1	3	Yes	No	No	Paralysis both hind legs	3†	Moderate	L1-2: 27 L3-4: 8 L5-6: 17	L1-2: 52 L3-4: 29 L5-6: 26	3.2
2	3	Yes	No	No	Paralysis both hind legs	1†	Moderate	T-L1: 10 L3-4: 7 L5-6: 28	T-L1: 37 L3-4: 20 L5-6: 16	2.2
3	3	No	No	No	None	8‡	Moderate	T-L1: 27 L3-4: 8 L5-S1: NA	T-L1: 38 L3-4: 0 L5-S1: 0	1.2
4	3	Yes	No	No	Severe paresis both hind legs, not ambulatory	4†	Mild	T-L1: 4 L3-4: 20 L6-S1: NA	T-L1: 21 L3-4: 40 L6-S1: 0	1.9
5	2	No	No	No	Paresis both hind legs, ambulatory	6	Moderate	L3-4: 34 L5-6: 37	L3-4: 85 L5-6: 17	3.1
6	2	Yes	No	No	Paresis both hind legs, ambulatory	6	Moderate	L2-3: 39 L4-5: 31	L2-3: 72 L4-5: 33	3.3
7	1	Yes	Yes	No	Delayed paresis of hind legs (day 6), ambulatory	7	Moderate	L4-5: 38	L4-5: 32	0.9
8	1	Yes	Yes	No	None	7	Severe	L5-6: 62	L5-6: 25	0.7
9	1	Yes	Yes	Yes	None	7	Severe	L2-3: 56	L2-3: 64	2.0
10	1	No	Yes	Yes	None	7	Moderate	L1-2: 43	L1-2: 58	1.8
11	1	Yes	Yes	Yes	None	7	Mild	L3-4: 22	L3-4: 34	1.0

† Clinical condition on day of termination, as this was prior to day 7 due to reaching the humane endpoint.
‡ Animal was terminated one day later to allow termination of the next animal in which the humane endpoint was reached.
NA: not applicable; SCC: spinal cord compression; DSP: dura sealant patch.

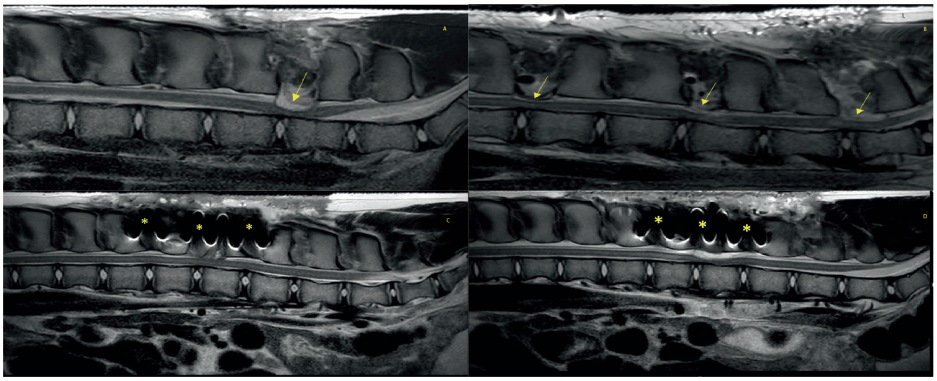

Figure 3. A) Postoperative MRI of the spine (pig #7) showing significant compression (arrow) of the spinal cord at level L4-5. B) Postoperative MRI of the spine (pig #1) showing compression (arrows) of the spinal cord at the three operated levels, i.e. last L1-L2, L3-4, and L5-L6. C and D) Postoperative MRI of the spine (pig #9 and #10) showing moderate compression on the operated level, L2-3 and L1-2 respectively. Artefacts (*) due to the screws used for fixating the Lubra plates still facilitate assessment of the spinal cord and dura.

Discussion

This article describes the learning curve of using a porcine spinal model with interlaminar decompression to test spinal medical devices. It was noted that fixation of the spine in a porcine *in vivo* model is feasible and may prevent neurological deficits. A total of 6 animals that had not received spinal fixation after decompressive laminectomy developed neurological deficits postoperatively, whereas none of the animals in which the spine was fixated showed postoperative neurological deficits.

To our knowledge, no other *in vivo* studies reported multilevel decompressive surgery in a porcine model. The high body weight of the pig model may be a predisposing factor to the assumed increased mobility of the spine after laminectomy, since a similar multilevel laminectomy model in dogs was not complicated by neurological deficits[12, 14]. Interlaminar decompression on multiple levels potentially destabilizes the porcine spine in an *in vivo* model, with severe neurological deficits as a result. Postoperative X-ray imaging of the spine to confirm this theory was, however, not performed in this study. Also, it remains inconclusive from the current study whether stability of the spine is maintained if ILD is restricted to one level, and if ILD is performed on multiple levels with fixation.

Spinal instability after decompressive surgery is a well-known problem and various surgical techniques have been developed to reduce destabilization. A biomechanical study in an *ex vivo* porcine model concluded that intervertebral displacement of the lumbar spine after laminectomy on one level is greater compared to bilateral

laminotomy[29]. Another study in an *ex vivo* porcine model showed that overall stability after muscle-preserving ILD on one level can be maintained[30]. A recent *in vivo* study to test an intradural spinal cord stimulation device in pigs showed successful recovery of 6 animals after simple one level laminectomy[19]. These results indicate that stability of the spine could be maintained after decompressive surgery on one level without fixation.

ILD on multiple levels with fixation of the spine has not been performed in this study, it is thus uncertain if surgery on multiple levels with fixation would be safe. The first 6 animals had interlaminar decompression on multiple levels (2 or 3), which resulted in neurological deficits in 5 out of 6 animals. Subsequently, two animals were operated on one level, yet neurological deficits occurred in one of these animals as well. Therefore, we performed an ILD on one level with fixation of the spine with lubra plates in the last 3 animals, which all recovered without deficits. No intra-operative complications occurred throughout the study. It was, however, apparent during the surgery that the durotomy led to severe decompression of the spinal cord. Although hemostasis was achieved during surgery, we believe the decompression of the spinal cord to have increased the risk for epidural hematoma postoperatively. The extent of compression was variable across animals operated on one or multiple levels and with and without fixation and did not seem to correspond well with neurological outcome. This is consistent with a previous study that finds no associated between SCC estimated on MRI and pre- or postoperative neurological status[26]. Thus, it may be hypothesized that the spinal cord injury occurred immediately after surgery when the animals awakened and tried to stand and walk, while their core spinal muscles were not completely functional. This may have resulted, also due to their high body weight, in vertebral subluxations at the laminectomy sites injuring the spinal cord and evoking myelopathy. Once the core spinal muscles regained their full tension, the spinal segment at the laminectomy site was stable again, leaving no evidence of spinal instability on MRI but resulting in myelopathy in all animals. Findings on MRI of hyperintensity of the spinal cord parenchyma on T2 in all animals confirmed this hypothesis. Since animal activity after surgery varied, this may have been one of the contributing factors to varying spinal cord compression on MRI. A study in dogs with presumed ischemic spinal myelopathy[28] shows that a lesion-length-to-vertebral-length ratio of >2.0 is 100% sensitive to predict unsuccessful neurological outcome. In our study 4 out of 6 pigs with neurological deficit had ratios >2.0. Whereas none of the animals without neurological deficit had ratios >2.0. The pigs with fixation of the spine showed a lesser (mean lesion-length-to-vertebral-length ratio 1.6 versus 2.0 in animals without fixation) extent of spinal myelopathy. The measurements of the length of hyperintensity of the spinal cord parenchyma on T2-weighted images are likely susceptible for high

interobserver variability as the transition from normal to abnormal spinal cord tissue is poorly defined.

Another factor that may have contributed to improved neurological outcome is the use of a wound drain. A wound drain was left in place for at least 24 hours in the last 5 animals which reduces compression of the spinal cord caused by postoperative oedema and wound fluid.

Although the biomechanical aspects of the native spine in quadrupeds have been studied in a previous study and did not seem to be different from the biomechanics of bipeds[5], the situation may be different for the quadruped spine after laminectomies. For this, experience is available in the veterinary literature, especially studies in dogs that undergo surgical procedures similar to humans for spinal disorders. Dogs that present with spinal lumbar fractures and spinal column instability after high impact forces [hit by car or fall from a height] are effectively treated by spinal fixation with Lubra plates to restore spinal stability, allow fracture healing and prevent secondary spinal cord injury[31]. The Lubra plates that were used in this study were also obtained from a veterinary company specialized in implants for companion animals. Fixation of the spine with pedicle screws and connecting rods has been performed successfully in canines[32], however the lubra plates allowed better postoperative imaging of the dura, which was necessary for the medical device test study this model was intended for. Furthermore, their limited availability for animal use and the high costs of human implants make this technique less suitable for a medical device test model.

Whilst our initial medical device study could not be completed as planned, the present study does lay the foundation for future porcine model studies for medical device testing. We have adapted the surgical technique according to the clinical outcomes and developed a feasible surgical porcine model. Furthermore, housing, handling, availability and societal acceptance of this species in research are favorable. Especially, the latter is an advantage over the use of a canine model for this purpose.

This study was limited by several factors. Firstly, this study was not designed to compare the neurological outcomes of different surgical techniques. Multiple factors have been altered throughout the study based on advancing insights to protect postoperative animal welfare as directed by the national ethical standards for animal experiments. Postoperative imaging was not included in the working protocol to evaluate the surgical technique (i.e. no X-ray was performed to assess spinal instability). Furthermore, two animals (#3 and #8) operated without fixation of the spine (one on multiple levels, another on one level) recovered without neurological deficit as well. Therefore, no definitive conclusions can be made based on these results. Secondly, a small number of animals was operated with fixation of the spine by Lubra plates. As the surgical

protocol was altered throughout the study, the spine was fixated with Lubra plates in the final three animals only. ILD on multiple levels with fixation of the spine has not been performed in this study, it is thus uncertain if surgery on multiple levels with fixation would be safe. Similarly, we only performed ILD on one level without fixation of the spine in two animals. Lastly, there was insufficient financial support to continue this study to further compare surgical techniques or the clinical outcomes related to the medical device this study was initially designed to test for.

This porcine model for ILD with fixation of the spine provides a useful basis for further preclinical research into the development of innovative surgical devices. In addition, sharing the lessons learned throughout the current study may contribute to reducing unnecessary animal suffering and research. Further research, with larger sample size, is necessary to evaluate our hypothesis that fixation of the spina in a porcine in vivo model of interlaminar decompression and durotomy improves neurological outcomes.

Data availability statement

The video recordings of the surgical procedures performed in this study are available upon reasonable request.

Conflict of interest statement

T.P.C. van Doormaal is a consulant for Polyganics B.V..

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Supplementary Material I
Definition of the human endpoint

The humane endpoint is defined as the occurrence of the following;

- Severe wound infection at the surgical site, severe suffering / weakness
- Severe neurological deficit with paralysis symptoms
- Postoperative inflammation / wound infection / temperature increase without improvement after adjustment of antibiotic treatment in consultation with the veterinarian or with severe suffering
- Severely reduced physical activity / withdrawn social behavior
- No eating for 48 hours postoperatively
- No drinking for 36 hours postoperatively
- Suffering of the animal, as assessed under article 13f (laboratory animal care taker), laboratory animal expert and veterinarian.

Supplementary Material II:
Quantifiable data of experimental animals

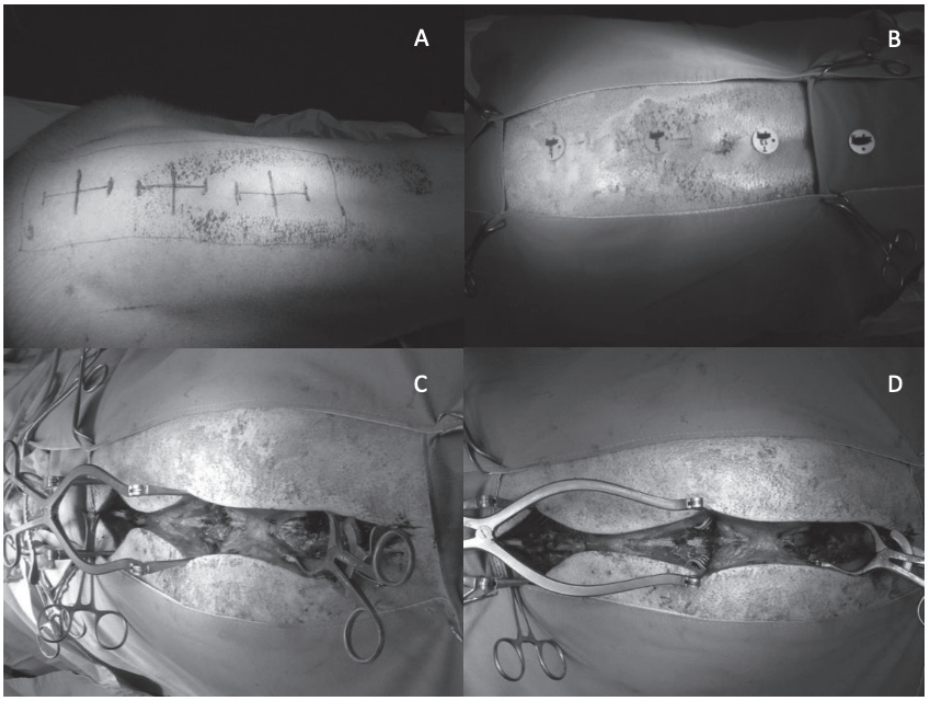

Figure 1. Perioperative marking of surgical levels in pig #1 and operative view before and after device implantation of pig#2

Note. Four steps of the operative process. Marking of the surgical levels is shown in panel A and B. The levels of interlaminar decompression before the implantation of the device and after application of the device are shown in panel C and D, respectively.

Table 1. Products used throughout study procedures.

Product	Manufacturer	Ref	Used in Pig #
Dura Sealant Patch (DSP)	Polyganics	DS01-024/08	1, 2, 4, 6, 7, 8, 9, 11
Bonewax	Ethicon	W31	4, 7, 8, 10, 11
Coated Vicryl 5-0 RB-1 Plus	Ethicon	V303H	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
Vicryl 2-0 FSL	Ethicon	V586	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
Vicryl 2 CTX Plus	Ethicon	V372	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
BMDS implantable programmable temperature transponder IPTT-300	BMDS		1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
Ray-cot 1.2” x 1.2”	American surgical company	W31	6, 7, 8, 9, 11
Lyostypt	B.Braun	1069128	2 (removed), 3 (removed), 4, 7, 8
Wound drainage system 40 ml CH6	Medinorm GmbH	5111006	7, 8, 9, 10, 11
Lubra plates (14.5 cm)	Vetinary Orthopedic Implants	E1903020	9, 10, 11
Screw with washer & nut for Lubra Plates (3/4 inch long)	Vetinary Orthopedic Implants	E193050B	9, 10, 11

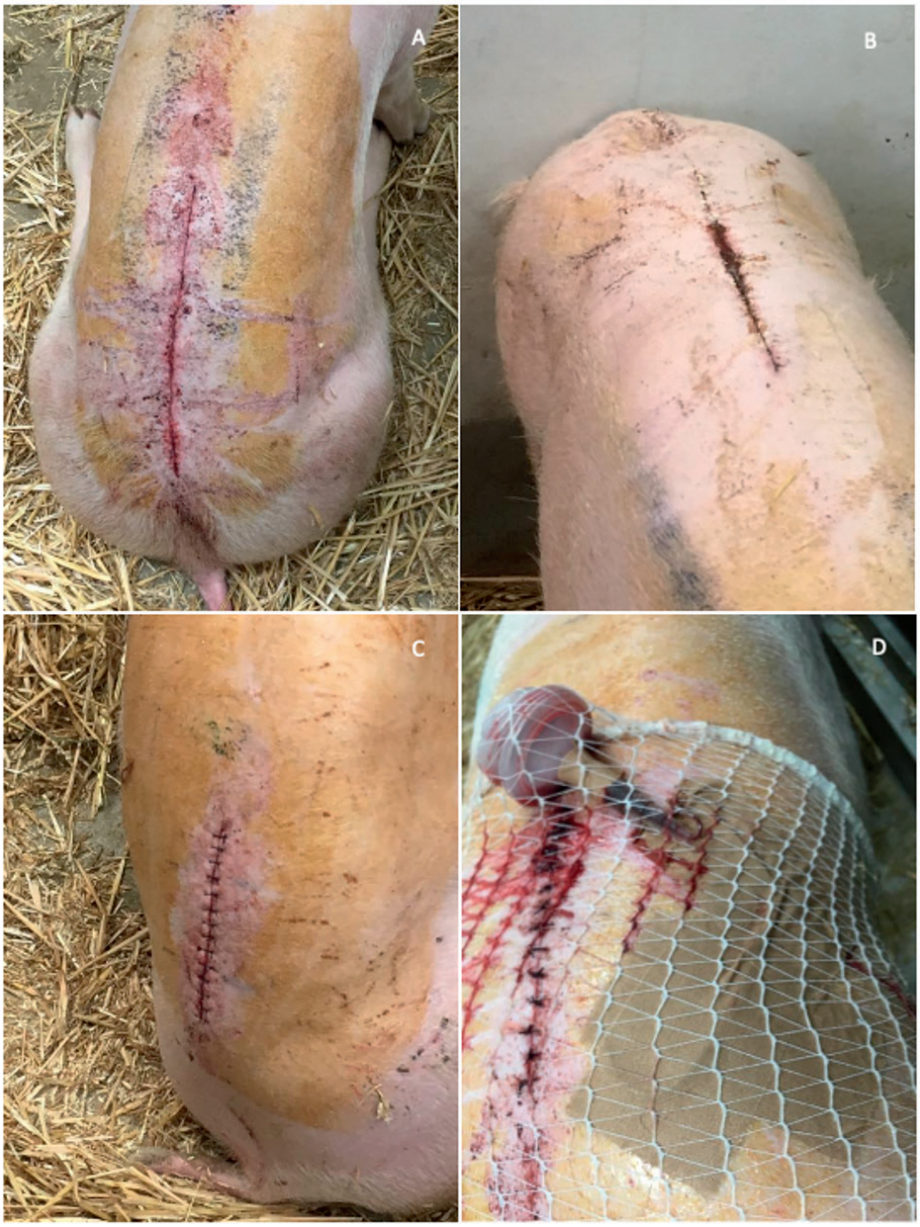

Figure 2. Postoperative view of the surgical wound in pig #1 (A) and pig #3 (B), pig # 5 (C) and pig # 8 (D).

Note. A wound drainage system was placed in pig 8 (panel D).

Table 2. Blood analysis results

Pig #	Experimental group	Survival time [Days]	Day 0 (Surgery) [x10^9/L]						Termination [x10^9/L]						Leukocytes Difference [[x10^9/L]]
			Leukocytes	Lymphocytes	Monocytes	Segments	eosinophils	basophils	Leukocytes	Lymphocytes	Monocytes	Segments	eosinophils	basophils	
01	DSP	3	**14.8**	9.7	0.4	4.5	0.2	0.0	**11.8**	7.1	0.3	4.2	0.1	0.0	**-3**
02	DSP	1	**18.6**	14.7	0.5	3	0.2	0.2	**28.4**	6.4	0.2	21.8	0.0	0.0	**9,8**
03	Control	8	**10.4**	6.7	0.1	3.5	0.1	0.0	**11.2**	6.8	0.2	4.1	0.1	0.0	**0,8**
04	DSP	4	**19.9**	15.3	0.4	3.7	0.3	0.1	**15.7**	11.3	0.5	3.6	0.2	0.1	**-4,2**
05	Control	6	**15.6**	8.7	0.2	6.4	0.2	0.1	**11.8**	5.3	0.2	5.8	0.4	0.3	**-3,8**
06	DSP	6	**19.2**	10.9	0.9	7.2	0.1	0.1	**20**	10.7	0.9	8.1	0.1	0.2	**0,8**
07	DSP	7	**13.8**	7.9	0.4	5.2	0.2	0.1	**20.5**	8.0	0.6	11.3	0.6	0.1	**6,7**
08	DSP	7	**14.6**	8.7	0.4	5.3	0.2	0.1	**15.6**	9.3	0.4	5.6	0.2	0.1	**1**
09	DSP	7	**12.9**	8	0.4	4.1	0.4	0.0	**14.8**	7.6	0.5	6.4	0.2	0.0	**1,9**
10	Control	7	**10**	3.2	0.3	3.4	0.0	0.0	**11.5**	6.3	0.6	4.6	0.0	0.0	**1,5**
11	DSP	7	**14.5**	9.7	0.5	4.0	0.1	0.1	**-***	-*	-*	-*	-*	-*	

* The blood sample of pig 11 at 7 days could not be processed due to clotting.

Supplementary Material III

Standardized observation scoring list

General condition	Good	0
	Fair	0
	Poor	0
Social behavior	Normal	0
	Withdrawn	0
Discomfort	None	0
	Little	0
	Moderate	0
	Severe	0
Wound (spine / back)	Dry	0
	Leakage	0
	Bloody	0
	Infection	0
	Explanation	0
Neurological sign	None	0
	Deficit	0
	Explanation	0

Supplementary material IV: Postoperative MRI images

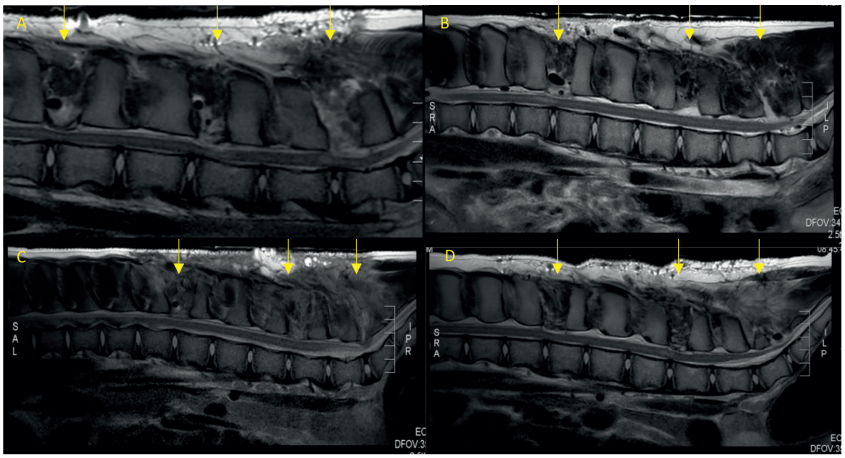

Figure 1. Postoperative sagittal MRI of pig #1, #2, #3 and #4 operated on 3 levels.

Note. A) Compression of the spinal cord is observed on all three operated levels (L1-2, L3-4, L5-6) in pig #1. B) Compression of the spinal cord is observed on all three operated levels (T-L1, L3-4, L5-6) in pig #2. C) Compression of the spinal cord is observed on all three operated levels (T-L1, L3-4, L5-S1) in pig #3. D) Compression of the spinal cord is observed on all three operated levels (T-L1, L3-4, L6-S1) in pig #4. The arrows indicate the operated levels.

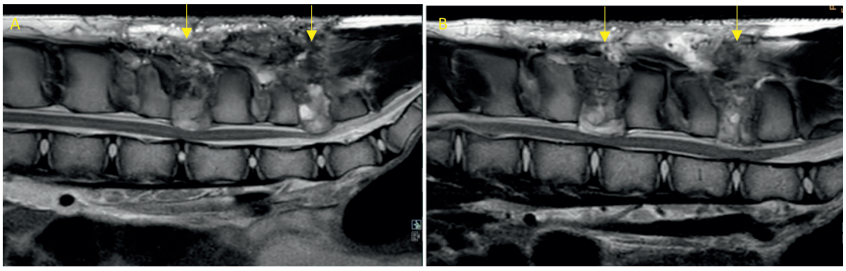

Figure 2. Postoperative sagittal MRI of pig #5 and #6 operated on 2 levels.

Note. A) Compression of the spinal cord is observed on both operated levels (L3-4, L5-6) in pig #5. B) Compression of the spinal cord is observed on both operated levels (L2-3, L4-5). The arrows indicate the operated levels in pig #6.

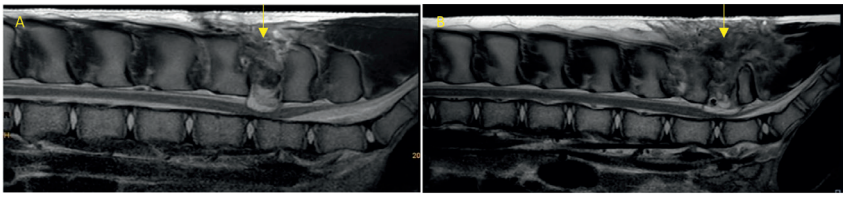

Figure 3. Postoperative sagittal MRI of pig #7 and #8 operated on 1 level.

Note. A) Compression of the spinal cord is observed on the operated level (L4-L5) in pig #7. B) Compression of the spinal cord is observed on the operated level (L5-L6) in pig #8. The arrows indicate the operated levels.

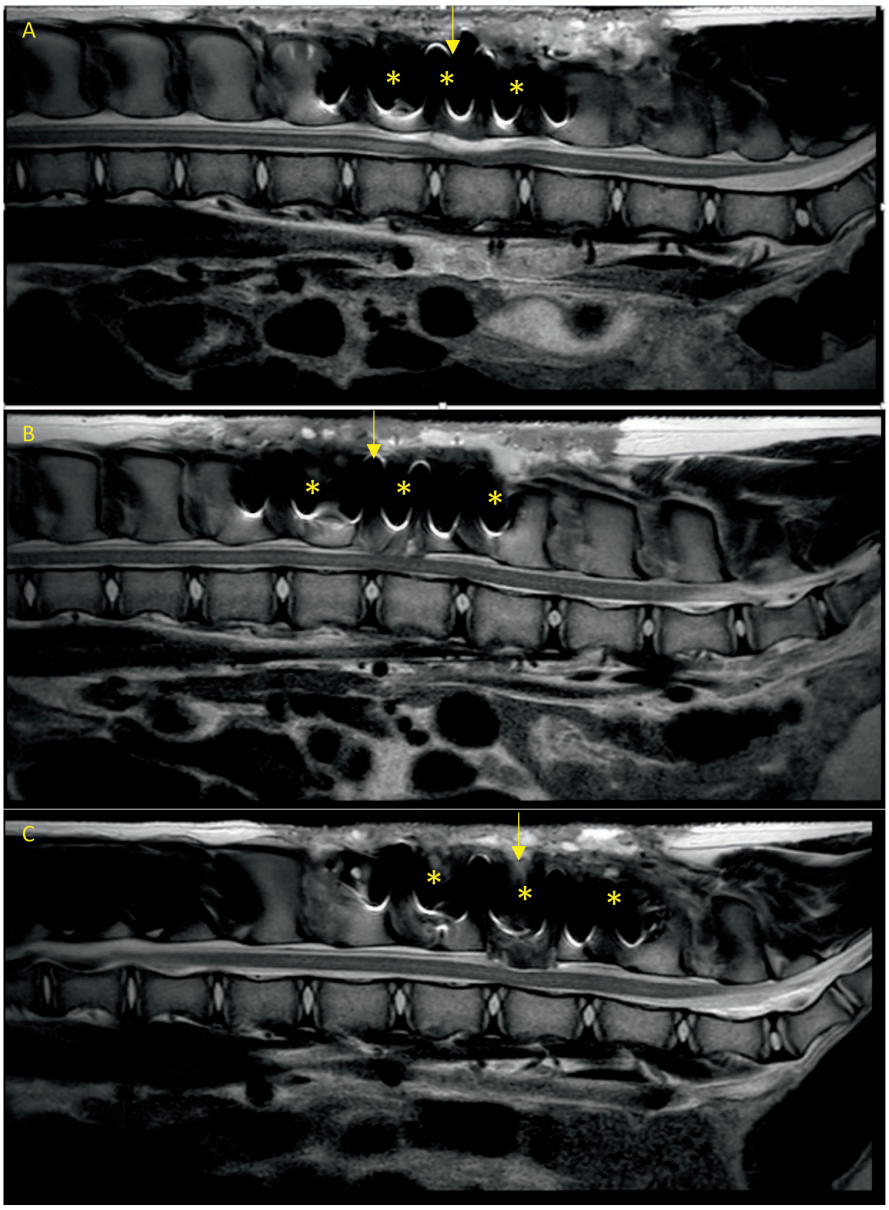

Figure 4. Postoperative sagittal MRI of pig #9, #10 and #11.

Note. A) Compression of the spinal cord is observed at the operated level (L2-L3) in pig# 9. B) Compression of the spinal cord is observed at the operated level (L1-L2) in pig #10. C) Compression of the spinal cord is observed at the operated level (L3-L4) in pig #11. Artefacts (*) due to the screws used for fixating the Lubra plates still facilitate assessment of the spinal cord and dura. The arrows indicate the operated level.


7

Histological and magnetic resonance imaging assessment of Liqoseal in a spinal in vivo pig model

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Animal Models and Experimental Medicine. 2022. doi: 10.1002/ame2.12294. Epub ahead of print.

Abstract

Liqoseal (Polyganics, B.V., Groningen, the Netherlands) is a dural sealant patch to prevent postoperative cerebrospinal fluid (CSF) leakage. It has been extensively tested preclinically and CE (*Conformité Européenne*) approved for human use after a first cranial in-human study. However, safety of Liqoseal with regard to spinal application is still unknown. The aim of this study was to assess safety of spinal Liqoseal application using histology and magnetic resonance imaging characteristics in comparison to cranial application.

Eight female Dutch Landrace pigs underwent laminectomy, durotomy with standard suturing and Liqoseal application. Three control animals underwent the same procedure without sealant application. The histological characteristics and imaging characteristics of animals of similar survival time were compared to data from a previous cranial porcine model.

Similar foreign body reaction was observed in spinal and cranial dura. The foreign body reaction consisted of neutrophils and reactive fibroblasts at up to 3 days, changing to a chronic granulomatous inflammatory reaction with increasing number of macrophages, lymphocytes and the formation of a fibroblast layer on the dura by day 7. Mean Liqoseal plus dura thickness had a maximum of 1.2 mm (range 0.7-2.0 mm) at day 7.

The spinal dural histological reaction to Liqoseal during the first 7 days was similar compared to the cranial dural reaction. Liqoseal does not swell significantly in both application areas over time. Given the current lack of a safe and effective dural sealant for spinal application, we propose that an in-human safety study with Liqoseal is the logical next step.

Abbreviations and Acronyms

CE - *Conformité Européenne*
CSF – cerebrospinal fluid
FDA – Food and Drug Administration
MRI – magnetic resonance imaging
USA – United States of America

Introduction

Cerebrospinal fluid leakage is a frequent complication after neurosurgical interventions, which is associated with prolonged hospital stay and increased healthcare costs[1,2]. To prevent CSF leakage, watertight closure of the dura mater is thought to be the most important step. Various products are used to augment this process, including approved sealants as well as off-label use of fibrin glues for this purpose[3]. Yet, their effectiveness has not been proven[4].

Therefore, a biodegradable synthetic dural sealant, Liqoseal, was developed (Polyganics B.V., Groningen, the Netherlands) (**Fig 1, chapter 3 page 44)**. The device consists of two layers; the watertight blue top layer is a biodegradable Poly(ester) ether urethane and the white bottom adhesive layer is made out of Poly(DL-lactide-co- ε-caprolactone copolymer and multiarmed NHS functionalized polyethylene glycol (PEG-NHS). Liqoseal is CE (*Conformité Européenne*) certified (2030288CE06) for cranial use since January, 2020.

Previous *ex vivo* experiments showed that Liqoseal provides a stronger watertight barrier than competitors in models mimicking cranial as well as spinal application situations[5]. However, Liqoseal is currently not approved for spinal use in humans. It is not clear if cranial results can be extrapolated to spinal application. Despite being a continuous membrane, there are differences between the spinal dura and cranial dura[6]. The spinal dura mater consists of the inner layer of the cranial dura mater, whereas the second, outer endosteal/periosteal layer of the cranial dura mater continues as periosteum on the level of the spinal cord[6]. The thickness of the dura mater is different at various levels along the spinal cord[6].

For this study we hypothesized that the acute (up to 7 days) dural reaction to Liqoseal on spinal porcine dura resembles the cranial porcine reaction. To evaluate this hypothesis we implanted Liqoseal spinally in 8 animals and compared histological results and thickness of dura plus sealant on magnetic resonance imaging (MRI) with the results of Liqoseal implantation in a cranial porcine *in vivo* model at similar survival times[8].

Materials and methods

This study has been approved by the animal experiment committee (DEC) Utrecht, the Utrecht Animal Welfare Body (IVD) and the Central Animal Experiments Committee affiliated to the Dutch National Institute for Public Health and the Environment (Approval No. AVD1150020184784 and AVD115002016457).

Intervention

The surgical model is described extensively in a previous publication, including anesthesiology protocol and learning points on spinal stabilization in pigs[7]. In short, a lumbar laminectomy and durotomy was performed in 11 female Dutch Landrace pigs of mean 78.3 (± 4.5) kg weight (**Figure 2A**). Dura was closed using interrupted sutures vicryl 5.0 (Ethicon, Somerville, United States of America (USA)). In 8 animals subsequently Liqoseal was applied (**Figure 2B**) and 3 were used as control. In the Liqoseal group a 2 by 1 cm piece of the sealant was cut and applied dry. Subsequently we applied manual pressure of approximately 1 kg using moist gauze for 2 minutes. Intraoperatively we did not perform further leakage tests to avoid disturbing the histological reaction and dural adherence. For the comparison to the cranial model study[8] we included 8 implantation animals with survival times similar to the cranial model, 3 who survived up to 3 (±1) days and 5 survived 7 (±1) days. Two control animals with spinal durotomy that survived 7 (±1) days were included (Supplementary Information I). The distribution of animals across survival groups is unequal due to initial postoperative complications which led to early termination of 3 animals. This was resolved by adjustments made to the surgical model for subsequent animals[7]. The planned survival time for this group was originally 7 days.

MRI assessment

Before termination, a spinal contrast enhanced MRI was made in prone position under anaesthesia. After the MRI the animals were euthanized with an overdose of pentobarbital. The thickness of the sealant combined with dural thickness was measured in Horos™, version 2.2.0. software on T2-weighted MRI without gadolinium in millimetres (mm) for the 8 spinal implantation animals. Thickness was measured at its maximum using the length tool at the first (most cranial) surgical level. When no clear distinction could be made between postoperative hematoma/oedema and the sealant combined with dura, no measurement was taken.

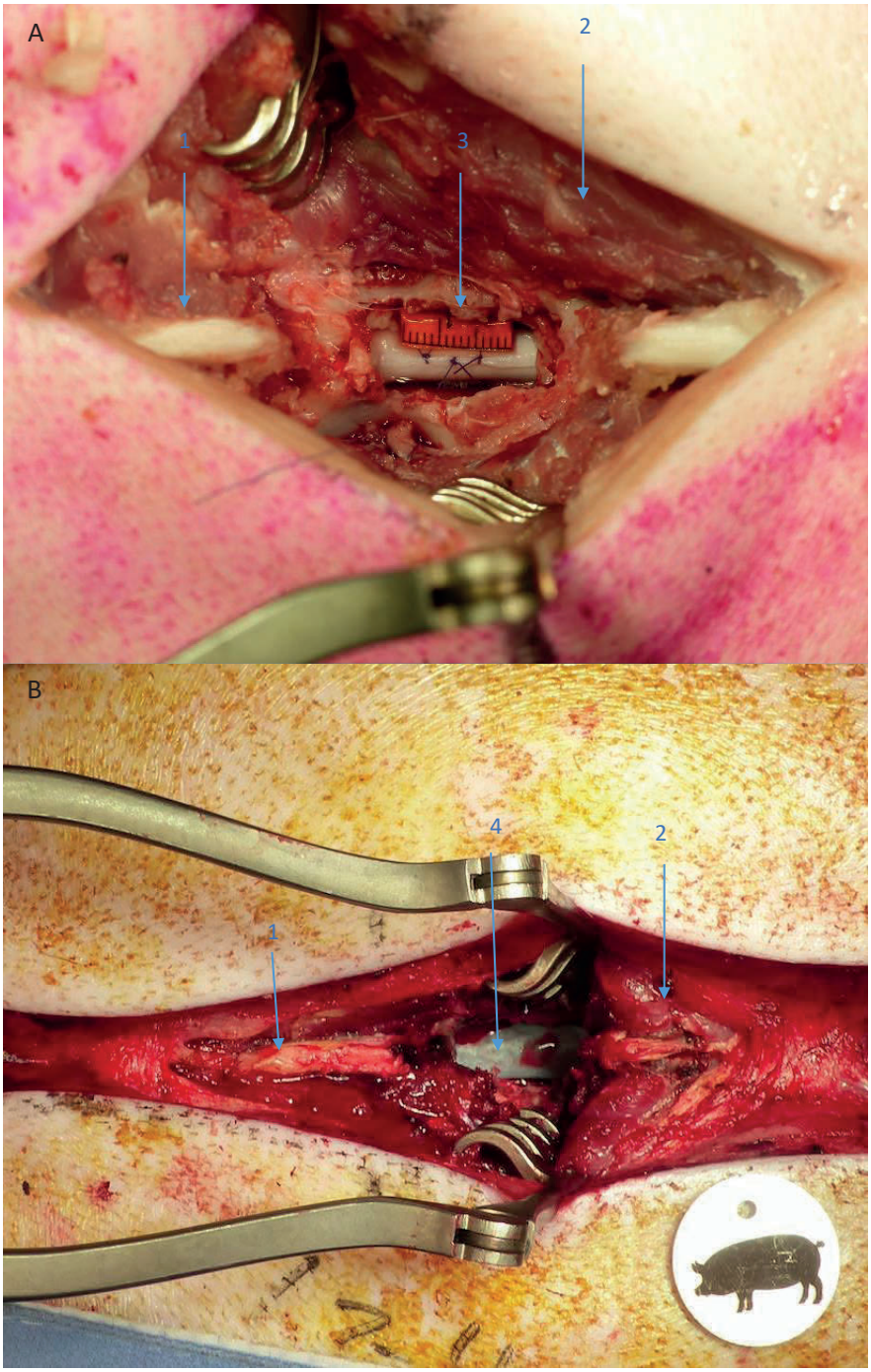

Figure 2. A) Dorsal view of interlaminar decompression on one level, showing the spinal cord (1) with sutured durotomy of 1.5 cm (3) and surrounding muscle tissue (2). B) Dorsal view of interlaminar decompression after the implantation of Liqoseal over the sutured durotomy (4).

Histological assessment

For histological analyses the operated region of the vertebral column was cut out *en bloc* with 1 cm margin around the surgical area and thus included the vertebra, appendicular process joints, skeletal muscle, meninges, spinal cord and spinal nerves.

The blocs were put in 10% neutral buffered formalin for fixation. After fixation, the sample was decalcified with Formical-4 (Statlab Medical Products Inc., McKinney, USA) at room temperature. On average, 14 days passed until the sample was decalcified. The decalcification process was evaluated daily by X-ray (Pathvision 23x29, Faxitron bioptics, LLC). After decalcification, the sample was routinely processed with use of isopropyl alcohol for dehydration and embedded in paraffin. Then slices of 0.4 mm were cut with a microtome and stained with haematoxylin and eosin.

Histological features scored were (1) inflammatory response, (2) necrosis, (3) neovascularization and (4) fibrosis. This analysis was performed by a board-certified veterinary pathologist (W.B).

Comparison group

In a previous study[8] the cranial reaction to Liqoseal was compared to Duraseal and Tachosil in a cranial *in vivo* model up to 12 months postoperatively. In this earlier study a total of 32 domestic pigs, of mean 66 (±5.7) kg weight, underwent craniotomy plus durotomy. This study showed that the foreign body reaction to Liqoseal is equivalent to these clinically used products at that time[8].

Histology and MRI results of the current study were compared with the data from this previous Liqoseal study in a cranial porcine model with similar survival groups to minimize the number of animals used. We included all animals with similar survival time (N=8) from this previous study as a comparison group. These 8 animals included 4 Liqoseal implantation animals, 2 survived 3 days and 2 survived 7 days. The other four pigs were control animals, of which 2 survived 3 days and 2 survived 7 days. An MRI was obtained on the day of termination[8].

For those cranial samples, the calvaria were cut out *en bloc* with a 1 cm margin around the bone flap and fixed in 10% neutral buffered formalin for one week. Thereafter coronal sections of 5-8 mm were created. Decalcification and processing for histological evaluation was performed as described for the spinal samples[8].

Results

Histology

In all 4 groups (spinal control, spinal Liqoseal, cranial control, cranial Liqoseal) the histologic reaction in all 4 categories ((1) inflammatory response, (2) necrosis, (3) neovascularization and (4) fibrosis) was similar **(Table 1)**.

Table 1. Overview of histological results

	Up to 3 (+-1) days	7 (+-1) days
Spinal		
Sealant	Hemorrhages Neutrophilic infiltration	Moderate amounts of macrophages Few multinucleated giant cells Moderately thick fibroblast layer
Dura	Mild acute inflammatory reaction; neutrophilic infiltration	Moderate subacute to chronic granulomatous inflammatory reaction
Cranial		
Sealant	Hemorrhages Neutrophilic infiltration Mild to moderate fibroblast proliferation Few multinucleated giant cells	Moderate subacute to chronic granulomatous reaction against the sealant. Moderate thick fibroblast layer.
Dura	Mild acute inflammatory reaction; neutrophilic infiltration Significant amounts of eosinophils Mild fibroblast proliferation	Moderate subacute to chronic granulomatous reaction Mildly thick fibroblast layer

Day 3 spinal

In the animals in the spinal group who survived up to 3 (±1) days the histological analysis showed haemorrhages and neutrophilic infiltration within the sealant **(Figure 3A)**. Within the dura a moderate predominantly neutrophilic infiltration was visible. At day 3 a mild fibroblast proliferation was seen.

No adhesion of the spinal cord to the dura mater nor the sealant was visible. Within the spinal cord either no changes or mild to severe Wallerian degeneration in the dorsal up to all funiculi was present. Occasionally also haemorrhages were present in the spinal cord.

Day 3 cranial

For the cranial group the reaction consisted of haemorrhages with mild to moderate neutrophilic infiltration within the sealant **(Figure 3B)**. Furthermore, multifocally bone spicules, caused by the creation of burr-holes during the cranial surgical procedure, were visible with a mild to moderate fibroblast proliferation with few macrophages and multinucleated giant cells. The multinucleated giant cells were occasionally seen within

the sealant. Within the dura mater there was predominant neutrophilic inflammation with a mild fibroblast proliferation. Additionally, significant amounts of eosinophils were visible. The histology in the cranial control animal was comparable to that in the animals with Liqoseal implantation.

No adhesions were visible between the nervous tissue and the dura mater or the sealant.

Both in control and Liqoseal pigs the underlying nervous tissue showed multifocally a mild lymphoplasmacytic meningitis, mild cortical oedema and moderate poliomalacia with demyelination of the corresponding white mater. Furthermore, occasionally a cell poor vasculitis was visible in the leptomeninges and cortex.

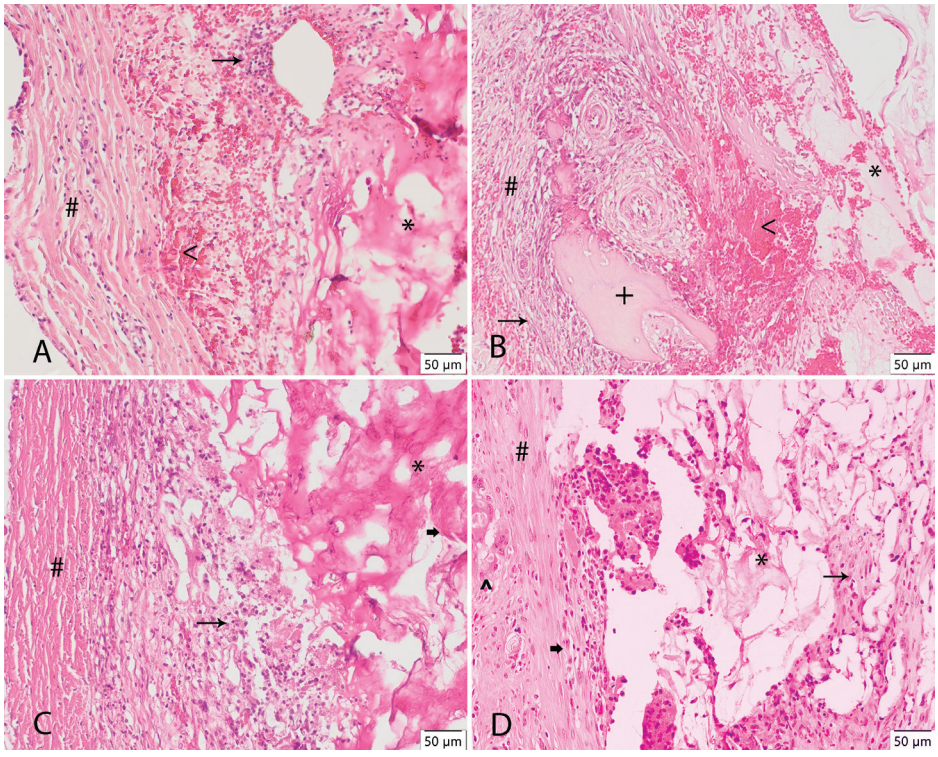

Figure 3. A) Pig with a spinal sealant, 3 days post- surgery. Between and in the dura mater and the sealant are haemorrhages (<) and neutrophils (→) visible. B). Pig with a cranial sealant, 3 days post-surgery. Between the dura mater and the sealant and in the sealant are haemorrhages (<) visible. The arrow points to fibroblast proliferation and macrophages surrounding a bone spicule (+). C) Pig with a spinal sealant, 7 days post-surgery. Between and in both the dura mater and the sealant is a granulomatous inflammation visible (→). D). Pig with a cranial sealant, 7 days post-surgery. Between the dura mater and sealant and within the sealant is a granulomatous inflammation visible (thin arrow). The thick arrow points to a layer of new formed fibroblasts. The arrow head (^) points to a granulomatous reaction directed to suture material. #, dura mater; *, sealant.

Day 7 spinal

In the spinal samples the number of macrophages increased with at 7 (±1) days moderate amounts of macrophages with presence of multinucleated giant cells (granulomatous inflammation) **(Figure 3C)**. At 7 days the fibroblast proliferation between the sealant and the dura mater started to become a moderately thickend fibrotic layer. The number of inflammatory cells in the dura and between the dura and the leptomeninges was small to moderate and changed from more acute with presence of neutrophils to a subacute to chronic infiltrate with lymphocytes, plasma cells and macrophages. In one Liqoseal pig suspected adhesion between the leptomeninges and the dura mater was seen. Within the spinal cord either no changes or mild Wallerian degeneration in different funiculi were present in both control and Liqoseal animals.

Day 7 cranial

The reaction in the cranial samples showed a distinct granulomatous inflammation redirected to the sealant. Furthermore, formation of a fibroblastic layer between the sealant and the dura mater was observed. Within the dura mater and between the dura mater and the leptomeninges again an inflammatory infiltrate shifting from a more acute to a subacute inflammation was visible **(Figure 3D)**. No adhesions of the cerebral tissue to either the dura mater or the sealant were visible. In both control and Liqoseal pigs multifocally a cell poor vasculitis with occasionally fibrin thrombi in both the leptomeninges and the cerebral cortex, a lymphoplasmacytic and histiocytic leptomeningitis with fibroblast proliferation, cerebral edema and poliomalacia and demyelination of the corresponding white matter was seen.

MRI

The sealant appeared hyperintense on T2-weighted images (**Figure 4A-D**). The combined thickness of dura and sealant was determined in 5 out of 8 animals in the spinal Liqoseal group. In 3 animals no clear distinction could be made between the sealant and postoperative oedema and hematoma.

Spinal MRI measurements in this study were not significantly different compared to earlier cranial measurements in a porcine model. The measured mean thickness of the sealant on MRI in all samples was 1.0 mm (range 0.7-2.0 mm). The mean thickness of the dura and sealant up to 3 (±1) days postoperatively was 0.8 mm (range 0.7-0.9 mm). The mean thickness of the dura and sealant at 7 (±1) days postoperatively was 1.2 mm (range 0.7-2.0 mm).

In the cranial model the mean thickness of dura and sealant was 0.9 mm (range 0.7 – 1.1) at 3 days and 1.1 mm at 7 days[8]. The overall mean thickness of dura and sealant was 1.0 mm (range 0.7-1.1) in the cranial model.

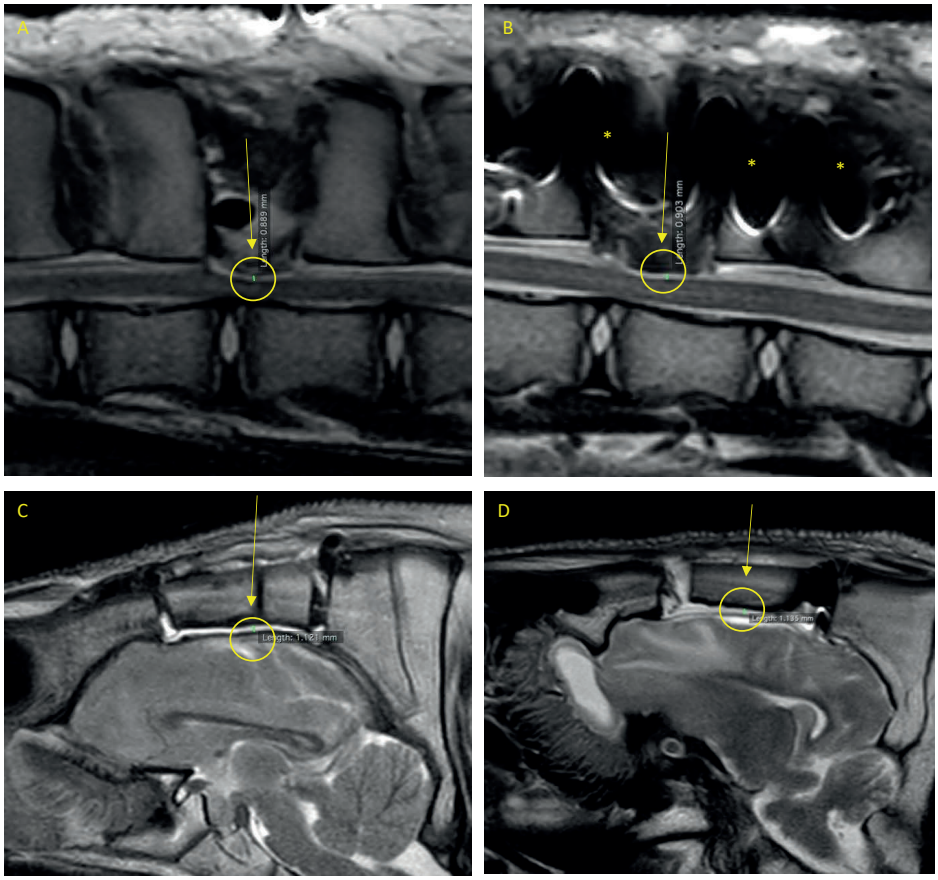

Figure 4. Sealant and dura thickness measurement on MRI indicated by arrows. A) Spinal group up to 3 days. B) Spinal group 7 days (artefacts (*) due to the screws used for fixating the Lubra plates (Veterinary Orthopedic Implants Inc, St. Augustine, USA) still facilitate assessment of the spinal cord and dura). C) Cranial group up to 3 days. D) Cranial group 7 days.

Discussion

We compared the foreign body reaction of spinal Liqoseal implantation to cranial implantation by combining histological and MRI assessments[8]. The histological reaction to Liqoseal observed in the spinal porcine *in vivo* model, is comparable to the reaction found in the cranial porcine *in vivo* model in the first 7 post-operative days. There was no indication of clinically significant swelling of Liqoseal and spinal dura up to day 7 (±1) postoperatively on MRI.

Over time we observed a foreign body reaction composed of neutrophils and reactive fibroblasts until day 3 (±1), changing to a subacute granulomatous inflammatory reaction with increasing number of macrophages, lymphocytes and the formation

of a fibroblastic layer on the dura by day 7 (±1) in the spinal model. This reaction was comparable to the cranial model, except for less pronounced presence of eosinophils and absence of multinucleated giant cells in the spinal model. This difference was probably caused by bone spicules present in the cranial samples which was caused by the creation of burr-holes and trepanation, as opposed to laminectomy performed with rongeurs.

In the previous cranial study which also included animals with longer survival times, Liqoseal appeared to be fully resorbed between 6 and 12 months compared to DuraSeal (Integra LifeSciences, Princeton, USA) and Tachosil (Corza Health, San Diego, USA), which were fully resorbed within 3 months[8]. The slower degradation properties of Liqoseal may allow the dural defect to heal completely while maintaining a watertight closure. The histological assessment of animals with longer survival times in the cranial study showed a decrease in inflammatory response from 1 months onwards, with only a minimal reaction present at 12 months[8]. Based on the similarities between the histological reaction in the spinal model in the short survival groups presented in the current study, we expect that the histological reaction will progress similarly to that presented in our previous cranial model with longer survival times[8].

The first in human single-arm trial ENCASE showed that Liqoseal is safe and easy to use in cranial surgery[9, 10]. None of the patients in this trial had intra- or postoperative CSF leakage. There was no indication of clinically significant swelling of the device on MRI imaging throughout the follow-up, comparable to the current study. At day 7 thickness is 3.5 mm (0.8-8.1 mm) and at 3 months it was 2.1 (0.8-7.4 mm), compared to a pre-implantation and compression thickness of 5 mm[9].

Swelling leading to spinal cord/nerve compression is a complication of concern with the use of DuraSeal which has been FDA (Food and Drug Administration) approved for spinal use. The hydrogel can swell up to 50% and cases of neurological deficit as a result have been reported[3, 11-14]. Similarly, this complication has been reported for off-label use of fibrin glue as well[15]. Thus, a sealant which does not swell after application has an important advantage. Swelling of the device, measured on MRI, should be an important safety measure in any future studies investigating the application of sealants in spinal surgery.

At this moment, there is no effective and safe sealant for spinal use available. Systematic review of the existing literature showed no significant difference in CSF leakage rate has been found between cases in which currently available sealants for spinal use were used in addition to suturing compared to cases with only primary suturing of the dura[4]. The CSF leakage rate in both groups was substantial at an average of 11%, while the secondary complications associated with CSF leakage are potentially life-threatening[4].

The current study is limited by the small sample size and short survival of the animals in the spinal *in vivo* model. This study was terminated before complete inclusion of the planned sample size and termination time for two reasons: 1. Model difficulties required various alterations to the surgical protocol throughout the study. 2. There was insufficient financial support to continue this costly study, with the adaptations that had to be made. Postoperative complications causing neurological deficit in the first animals required their early termination. Adaptations to the surgical model, with fixation of the spine, resolved this issue allowing for complete survival of 7 days in subsequent animals[7]. For these reasons the distribution of intervention versus control animals across the groups of varying survival time are skewed.

In addition, the measurements of the sealant and dura thickness on MRI could be performed on a limited number of animals, because of the difficult distinction between sealant/dura on MRI and postoperative oedema or hematoma in some cases. This limited sample size does not allow for statistical comparison of the measurements between groups.

Despite the limited sample size of the spinal *in vivo* study, we believe that the results of the current study and its comparison to the previously published cranial *in vivo* studies provides valuable evidence for the use of Liqoseal in spinal surgery. The comparison of data from the current spinal model to a previously published cranial model contributes to reducing unnecessary animal research. Preclinical safety data for future spinal in human trials may be obtained from these results instead of setting up a larger spinal animal study with longer survival.

In conclusion, the spinal dural histological reaction to Liqoseal during the first 7 days was similar compared to the cranial dural reaction. Liqoseal does not significantly swell in both application areas over time. Furthermore, no safety issues were reported in the first in human cranial study (ENCASE)[9].

Combined with previous data, this study suggests that Liqoseal can be safely applied on spinal dura. Given the current lack of safe and effective dural sealant for spinal surgery and burden of disease caused by CSF leakage, we propose that an in-human study investigating safety and efficacy of Liqoseal in spinal surgery is the logical next step.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Tristan van Doormaal, Bart de Boer, Wilhelmina Bergmann, Saskia Redegeld and Sander van Thoor, Ahmet Kinaci and Emma Slot. The first draft of the manuscript was written by Emma Slot and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest:
TPCvD is a consultant for Polygancis b.v, AK received a research grant through Polyganics b.v., EMHS receives a research grant through Polyganics b.v. The other authors report no conflicts of interest.

Acknowledgement
None.

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IV

Cerebrospinal fluid leakage in children

Cerebrospinal fluid leakage after cranial surgery in the pediatric population – A systematic review and meta-analysis

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Child's Nervous System. 2021;37(5):1439-1447.
doi: 10.1007/s00381-021-05036-8.

Abstract

Cerebrospinal fluid (CSF) leakage is a common complication after neurosurgical intervention. It is associated with substantial morbidity and increased healthcare costs. The current systematic review and meta-analysis aims to quantify the incidence of cerebrospinal fluid leakage in the pediatric population and identify its risk factors.

The authors followed the PRISMA guidelines. The Embase, PubMed and Cochrane database were searched for studies reporting CSF leakage after intradural cranial surgery in patients up to 18 years old. Meta-analysis of incidences was performed using a generalized linear mixed model.

Twenty-six articles were included in this systematic review. Data were retrieved of 2,929 patients who underwent a total of 3,034 intradural cranial surgeries. Surprisingly, only four of the included articles reported their definition of CSF leakage. The overall CSF leakage rate was 4.4% (95% CI 2.6 to 7.3%). The odds of CSF leakage were significantly greater for craniectomy as opposed to craniotomy (OR 4.7, 95% CI 1.7 to 13.4) and infratentorial as opposed to supratentorial surgery (OR 5.9, 95% CI 1.7 to 20.6). The odds of CSF leakage were significantly lower for duraplasty use vs. no duraplasty (OR 0.41 95% CI 0.2 to 0.9).

The overall CSF leakage rate after intradural cranial surgery in the pediatric population is 4.4%. Risk factors are craniectomy and infratentorial surgery. Duraplasty use is negatively associated with CSF leak. We suggest to define a CSF leak as “leakage of CSF through the skin”, as an unambiguous definition is fundamental for future research.

Key Words

Cerebrospinal fluid leakage, craniotomy, craniectomy, posterior fossa surgery, pediatrics

Introduction

Cerebrospinal fluid (CSF) leakage is one of the most common complications after neurosurgical intervention. CSF leakage is associated with substantial morbidity and increased healthcare costs.[1] One study found an average cost difference of €17.412 for patients with postoperative CSF leakage compared to patients without CSF leakage.[1] CSF leakage may lead to the development of a pseudomeningocele (PMC), wound healing problems requiring surgical re-closure, surgical site infection, meningitis and pneumocephalus. CSF leakage rates reported in pediatric studies range between 0-38%.[2-6] Definitions of CSF leakage vary in the existing body of literature.

The exact magnitude of the problem in children, however, is still unknown and may be larger than in adults for several reasons. First, almost half of all pediatric brain tumors resides in the posterior fossa and posterior fossa surgeries are thought to be more prone to CSF leakage.[1, 7, 8] Second, intraventricular tumors are more common in the pediatric population.[8] Surgical opening of the ventricle may result in higher chance of postoperative CSF leakage.[9] A clear understanding of the incidence and risk factors of CSF leakage in the pediatric population is essential in the prevention of CSF leakage in children. The current systematic review and meta-analysis aims to address these issues.

Methods

The authors followed the PRISMA guidelines for this systematic review and meta-analysis.[10]

Search strategy and selection criteria

Embase, PubMed and Cochrane databases were searched until August 31st, 2020 for studies reporting CSF leakage and related complications after intradural cranial surgery in patients up to 18 years old. The following search terms were used: “children” OR “child” OR “pediatric” OR “paediatric” OR newborn OR “adolescent” OR “infant” AND ”neurosurgery” OR ”craniotomy” OR “craniectomy” OR “cranial surgery” OR “tumor resection” AND ““cerebrospinal fluid leakage” OR “CSF leakage” OR “pseudomeningocele” OR “incisional leakage” OR “wound leakage” OR “surgical site infection” OR “surgical wound infection” OR “meningitis”” and relevant Mesh/Emtree terms. A modified version of the filter used to search pediatric studies in PubMed is used (see Appendix 1-3 for the full search strings).[11] Studies written in other languages than English, Dutch, German, French, Italian or Spanish were excluded. Studies written before 1966 were excluded, as those are not included in the PubMed database. Laboratory studies, animal studies, cadaveric studies, case reports, small case series (N<10) and literature reviews were excluded. Furthermore, studies on transsphenoidal surgery, skull base reconstructions, burr hole surgery (i.e. drainage of chronic subdural hematoma, needle biopsy) and

primary CSF diversion surgeries were excluded. Two authors (EMHS and KMvB) independently screened all records from the database search on title and abstract to identify relevant articles. All remaining full text articles were screened on their eligibility for inclusion. A consensus meeting was held to reach agreement on the included articles.

Data extraction

The following patient specific data items were extracted as proportion or mean per study: age, gender, compromised immune status, previous chemotherapy or radiotherapy, presence of hydrocephalus preoperatively, CSF diversion surgery (endoscopic third ventriculostomy (ETV)/external ventricle drain (EVD)/ ventriculoperitoneal (VP) shunt). The following surgical items were collected as proportion per study: site of durotomy (infratentorial/supratentorial), craniotomy versus craniectomy, indication for surgery (i.e. tumor resection or Chiari decompression), ventricular opening (yes/ no), use of sealant (yes/ no), use of duraplasty (yes/no), and whether a "watertight" closure of the dura was attempted or not. The following outcome parameter was collected: proportion of patients with CSF leakage (based on the individual study's definition).

Study quality was assessed according to the National Heart, Lung and Blood Institute of National Institutes of Health (NIH) quality assessment tool for case series studies.[12] Studies with more than 2 items with high risk for bias or unclear risk for bias were classified as poor quality. Studies with a maximum of 2 items with high risk for bias or unclear risk for bias were judged to be of fair quality. Studies with no items with high risk of bias and a maximum of 1 item with unclear risk of bias were deemed of good quality.

Statistical analysis

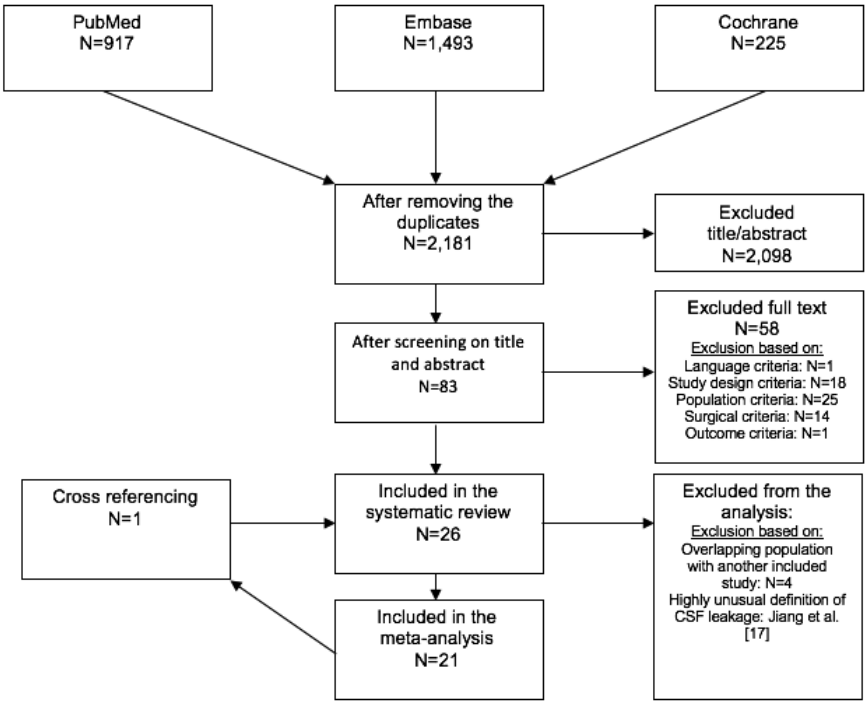
A meta-analysis of the incidence of CSF leakage was performed using a generalized linear mixed model. Heterogeneity of the data across studies was determined using Higgins I^2.[13]

The primary outcome measure in this study is the incidence of CSF leakage with 95% confidence interval (CI). Subgroup analyses were performed for the separate surgical indications Chiari decompression (with dural opening) and posterior fossa tumor surgery. Secondary outcome measures are the odds ratio (OR) for CSF leakage for craniotomy versus craniectomy, supratentorial versus infratentorial surgery, cases in which a duraplasty was used or not and studies in which watertight closure was attempted in all cases or not. Finally, three sensitivity analyses were performed 1) for studies of high quality only, 2) for studies of >50 patients only, 3) including the study of Jiang et al (see Results section).[4]

All analyses were performed using SAS version 9.4 (SAS Institute Inc).

Results

The database search yielded 2,123 articles of which 26 were included in this systematic review **(Figure 1)**. Twenty-one articles were included in the meta-analysis, as four articles had to be excluded because of overlapping study populations (the article discussing the largest sample size was included).[6, 14-16] Additionally, the study of Jiang et al. was excluded from the meta-analysis, because they unconventionally diagnosed CSF leak when 'drainage from the drainage catheter was clear and transparent' in their patient population in which placement of a low-vacuum suction wound drain was part of the surgical protocol.[4]



Legend
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Figure 1. Flowchart of study selection

A total of 2,929 patients were included, who underwent a total of 3,034 intradural cranial surgeries, as some patients had more than one surgery. **Table 1** provides an overview of study characteristics.

Table 1. Overview of included studies

Author	Study design	Surgery type	Definition of CSF leakage	Population (N)	Surgeries (N)	Age (yrs), mean	Age (yrs), range	CSF leakage incidence (%)	Study quality
Cochrane et al., 1994[a, 14]	CCS	Posterior fossa tumor		91	91	7.3	0.20-16	4.4	Poor
Culley et al., 1994[21]	CCS	Posterior fossa tumor		117	117		0.3-16	19.7	Poor
Muszynski et al., 1994[18]	CCS	Posterior fossa tumor	CSF visibly dripping from the surgical wound	50	50		2-13	14.0	Good
Parizek et al. 1998[26]	CCS	Posterior fossa tumor		454	439			4.6	Poor
Gnanalingham et al., 2002[30]	CCS	Posterior fossa tumor		110	110	5.8	0.2-15	15.5	Fair
Gnanalingham et al, 2003[b, 15]	CCS	Posterior fossa tumor		84	84	5.8	0.5-16	10.7	Fair
Bognar et al., 2003[20]	CCS	Posterior fossa tumor		180	180	7.4	0.3-16	7.2	Poor
Steinbok et al., 2007[19]	CCS	Posterior fossa tumor	CSF leak through the skin	154	174			10.3	Good
Gopalakrishnan et al., 2012[22]	CCS	Posterior fossa tumor		84	84	8.0	1.5-18	2.4	Poor
Panigrahi et al., 2012[5]	RCT	Posterior fossa tumor		14	14	8.1	2-15	0.0	Poor
Hale et al, 2019[23]	CCS	Posterior fossa tumor		186	186	7.6	3.4-12	8.1	Fair
Kushel et al., 2019[25]	CCS	Posterior fossa tumor		211	211			5.2	Fair
Houdemont et al., 2011[24]	CCS	Craniotomy for tumor		99	117	7.4		6.8	Poor
Lassen et al., 2011[c, 16]	CCS	Craniotomy for tumor	All CSF leaks and pseudomeningoceles requiring surgical intervention	211	273	8.5	0-18	7.3	Good
Hosainey et al., 2014 [17]	CCS	Craniotomy for tumor	All CSF leaks and pseudomeningoceles requiring surgical intervention	302	381	8.6	0-18	6.3	Good
Krieger et al., 1999[31]	CCS	Chiari decompression		31	31		0.5-18	9.7	Fair
Parker et al., 2011[27]	CCS	Chiari decompression		114	114	8.6		5.3	Fair
Hidalgo et al., 2018[3]	CCS	Chiari decompression		105	105	10.0		0.0	Fair
Jiang et al., 2018[d, 4]	RCT	Chiari decompression		42	42	14.0	10-18	38.1	Fair

Table 1. *(continued)*

Author	Study design	Surgery type	Definition of CSF leakage	Population (N)	Surgeries (N)	Age (yrs), mean	Age (yrs), range	CSF leakage incidence (%)	Study quality
Zhou et al., 2014[38]	CCS	Cranial surgery		163	160	10.2		1.3	Poor
Roth et al., 2018[28]	CCS	Craniotomy		157	163		0.3-18	0.6	Fair
Soleman et al., 2019[e, 6]	CCS	Interhemispheric approach for various intracranial pathologies		26	28	10.1	2-17	0.0	Fair
Vedantam et al., 2017[29]	CCS	Craniotomy for epilepsy		280	280		4-13	0.4	Fair
Srinivasan et al., 1999[33]	CCS	Bifrontal olfactory nerve-sparing craniotomy		14	14	8.6	2-18	7.1	Poor
Levy et al., 2003[32]	CCS	Microsurgical keyhole approach for middle fossa arachnoid cyst fenestration		50	50	5.7	0.1-17	6.0	Fair
Dlouhy et al., 2015[2]	CCS	Supraorbital Eyebrow craniotomy		54	54	9.6	1.5-16	0.0	Fair
Total[f]				**2,929**	**3,034**				

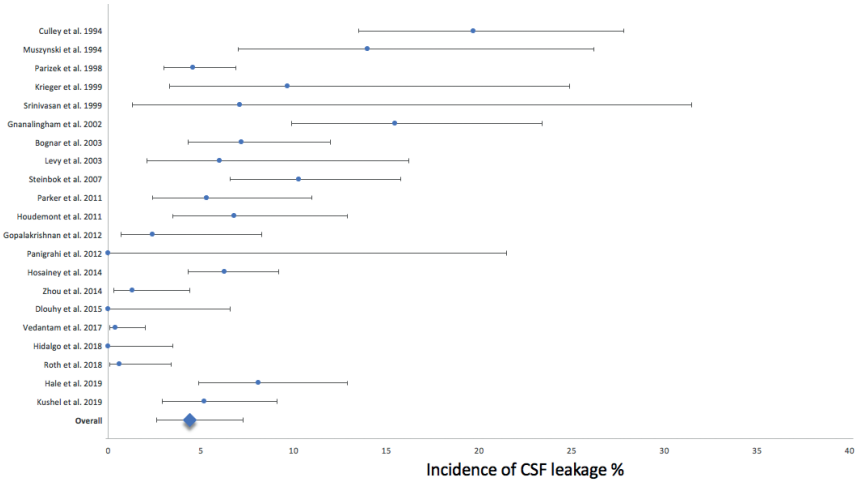
Legend

[a] Excluded from meta-analysis because of overlap with Steinbok et al., 2007
[b] Excluded from meta-analysis because of overlap with Gnanalingham et al., 2002
[c] Excluded from meta-analysis because of overlap with Hosainey et al., 2014
[d] Excluded from meta-analysis because of overestimation of CSF leakage resulting from wound drainage and inclusion of clear production in the drainage system as CSF leakage
[e] Excluded from meta-analysis because of overlap with Roth et al., 2018
[f] Studies included in the meta-analysis only
N number
CSF cerebrospinal fluid
yrs years
CCS Consecutive case series
RCT Randomized controlled trial

Most included articles report retrospective consecutive case series. One study was a randomized controlled trial, in which patients were randomized for a crescent incision versus a Y-shaped incision of the dura.[5] Ten studies were of poor quality, based on unclear description of the surgical procedure and poor definition of the outcome measure CSF leakage and either insufficient reporting of the follow up duration or lack of description of statistical methods. Twelve studies were of fair quality, again largely based on a lack of adequate definition of the outcome measures and inadequate reporting of statistical methods. Four studies were of good quality, these studies all provide a clear definition of the outcome measure CSF leakage. A detailed description of the quality assessment is presented in Supplementary Information 1.

Primary outcome measure

The overall incidence of CSF leakage was 4.4% (95% CI 2.6 to 7.3%) **(Figure 2)**.



Legend

CSF Cerebrospinal fluid

Figure. 2 Forest plot incidence of CSF leakage

Subgroup analyses for type of surgery could only be performed for Chiari decompression (with dural opening) and posterior fossa tumor surgery, as only these indications were investigated in sufficient studies. CSF leakage rates in these subgroups were 3.4% (95% CI 1.3% to 8.7%) after Chiari decompression, and 8.0% (95%CI 5.2-12.0%) after posterior fossa tumor surgery. All analyses showed substantial heterogeneity. An overview of outcomes for the primary outcome measure and subgroup analyses can be found in **Table 2**.

Table 2. Incidence of CSF leakage based on generalized linear mixed model

Outcome	Incidence (%)	Lower bound (%)	Upper bound (%)	Std Error	I^2	Studies (N)	Surgeries (N)
Overall	4.4	2.6	7.3	1.1	93.6	21	3,034
Posterior fossa tumor resection	8.0	5.2	12.0	1.7	87.8	10	1,545
Chiari decompression	3.4	1.3	8.7	1.7	58.5	3	250

Legend
CSF Cerebrospinal fluid

Secondary outcome measures

The highest percentage of CSF leakage was found in patients undergoing craniectomy (10.3%, 95% CI 4.3% to 22.7%), with an OR of 4.7 (95% CI 1.7 to 13.4) compared to craniotomy (2.4%, 95% CI 1.0% to 5.4%). A CSF leakage rate of 6.4% (95% CI % 4.1 to 10.0%) was found for infratentorial surgery in contrast to 1.2% (95% CI 0.4 to 3.7%) for supratentorial surgery (OR 5.9, 95% CI 1.7 to 20.6).

In patients with a duraplasty for dural closure the incidence of CSF leakage was 5.3% whereas patients without a duraplasty had a significantly higher incidence of 11.8% (OR 0.41, 95% CI 0.19 to 0.90).

In studies in which watertight closure was attempted in all cases, the CSF leakage rate was 2.3% as compared to 6.4% patients in studies in which watertight closure was not attempted in all cases (OR 0.34 95% CI 0.1 to 2.3). An overview of the secondary outcome measures is presented in **Table 3**.

Table 3. Overview secondary outcome measures

Outcome	Odds ratio	Lower bound	Upper bound	P-value	Studies (N)	Surgeries (N)
Craniectomy vs. Craniotomy	4.7	1.7	13.4	0.00*	15	1,917
Infratentorial vs. Supratentorial	5.9	1.7	20.6	0.01*	18	2,373
Duraplasty vs. no duraplasty	0.4	0.2	0.9	0.03*	5	727
Watertight closure in all cases vs. watertight closure not in all cases	0.3	0.1	2.3	0.27	10	1,415

Legend
CSF Cerebrospinal fluid
* Significant

Sensitivity analysis

Separate analyses were performed: 1) for studies of high quality only, 2) for studies of >50 patients only, 3) including the study of Jiang et al..[4] The overall CSF leakage rate in studies of good quality is 7.4% (95% CI 4.6 to 11.6%).[17-19] For studies of more than 50 patients the CSF leakage rate was 3.8% (95% CI 2.0 to 7.3%).[2-4, 17, 20-29] The meta-analysis including the study of Jiang et al. results in an overall CSF leakage rate of 4.8% (95% CI 2.7% to 8.3%).[4] An overview of outcomes for the sensitivity analyses can be found in Supplementary Information 2.

Discussion

This meta-analysis shows that the overall incidence of CSF leakage after intradural cranial surgery in the pediatric population is 4.4%. Infratentorial as opposed to supratentorial surgery, and craniectomy as opposed to craniotomy are significant risk factors for CSF leakage (OR 5.9 and 4.7, respectively). These results underline the relevance of CSF leakage in clinical practice. In the pediatric population, specifically, the burden of additional treatment that may be required for CSF leakage or related complications is substantial. In studies reporting data on treatment of CSF leakage a total of 37 out of 114 patients with a CSF leak were treated with a ventriculoperitoneal shunt.[17, 19, 20-22, 25, 30-33]

There is a wide range of reported CSF leakage rates (between 0.0% and 38.0%).[4,5] This may have several reasons. First, there is a large variability in the definition of CSF leakage. Moreover, only four out of 26 studies actually described their definition of CSF leakage. Secondly, the wide incidence range may be due to the different types of surgery included across studies (i.e. supra orbital eyebrow craniotomy, epilepsy surgery, posterior fossa tumor surgery).

No separate analyses could be performed per type of surgery for these categories, nor for the risk factors *age, immune status, previous chemotherapy* or *previous radiotherapy, CSF diversion surgery, preoperative hydrocephalus, ventricular opening* and *sealant use* as there was insufficient data or only data on study level available from the included literature.

Our meta-analysis shows that the proportion of CSF leakage is highest in the subgroup of patients undergoing craniectomy (10.3%). This difference may be explained by the lack of extra counter pressure that is otherwise provided by the replaced bone flap.[30] Replacement of the bone flap decreases the continuous short increase and decrease in dural stress caused by the triphasic pulsations of cerebrospinal fluid.[34] Furthermore, the bone flap may reduce the dead space which is created after detachment of the muscles in the suboccipital region and support their reattachment to the replaced

bone flap, so that collection of CSF in this space is limited and pseudomeningocele is prevented.[30]

This meta-analysis finds a CSF leakage rate of 3.4% after Chiari decompression surgery. The relatively low leakage rate in this population is surprising considering the above-mentioned surgical risk factors (infratentorial surgery and craniectomy) as this population essentially represents a combination of these two items.

On the contrary, a high leakage rate in posterior fossa tumor surgery (8.0%) is found. This type of surgery may be prone for leakage because pediatric brain tumors frequently reside in the fourth ventricle, requiring opening of the telovelar membrane and leaving a wide-open ventricle. Furthermore, postoperative hydrocephalus may contribute to the increased incidence of CSF leakage in this population.[23]

The effect of watertight closure was not significant in this study. However the effect in this analysis may be limited because it was only possible to compare studies in which all cases were closed in watertight fashion to those in which not all cases were closed with this aim (the dura was left open in all cases in one study[31], in other studies 10-89%[18, 26, 28] of cases were not closed in a watertight manner).

CSF leakage was significantly less frequent in patients in whom a duraplasty was performed (OR 0.41). This may reflect that when careful attention is paid to optimal closure of the dura with or without augmentation such as duraplasty or sealants the risk of CSF leakage is reduced. No distinction has been made in this study between autologous or synthetic material. A study by Hale et al. (2020) indicates that graft dural closure may furthermore be protective against hydrocephalus and wound infection in patients undergoing posterior fossa tumor surgery.[23]

Compared to adults the incidence of CSF leakage found in children is considerably lower, which is contrary to our expectations considering the high number of craniectomies and infratentorial surgeries included. A recent meta-analysis has found that the rate of CSF leakage in adults is 8%.[35] As is the case in pediatric literature, the definition of CSF leakage reported in studies on adults is not uniform either. This may explain the discrepancy between the incidence of CSF leakage in both populations. Another factor may be that the meta-analysis on adults includes studies in which sealants use was compared, this patient population may, therefore, be one which is more prone to CSF leakage, considering a substantial number of studies selected patients based on intraoperative CSF leakage. Moreover, this may be a result of increased flexibility of the tissues in children compared to adults allowing for better surgical closure of the dura and skin layers.

This meta-analysis is subject to several limitations. Most importantly, the studies included are heterogenous in their definitions of the outcome measure, population and follow-up

duration. The majority of studies included in this meta-analysis do not clearly define the outcome measure CSF leakage. Those that do, use a variety of definitions, for example, being 'CSF leak through the skin' and 'all CSF leaks requiring surgical intervention'.[17, 19] This obviously results in differences in outcome, as is reflected by the I^2-values found in the meta-analyses. It was not possible to adopt a specific definition of CSF leakage for this meta-analysis, as too few publications mention this. One study has been excluded because it included clear fluid in a low-vacuum suctioning wound drainage system as CSF leakage, resulting in an outstandingly high CSF leakage rate of 38.0%.[4] In a sensitivity analysis including this publication we found an overall CSF leakage rate of 4.8% (4.4% without), indicating this study has no clinically meaningful influence on the overall outcome.

Secondly, the risk factor analyses for duraplasty use and watertight closure were based on a limited number of studies. Therefore, caution should be applied in generalizing these results.

Thirdly, we did not exclude patients with subdural-to-extracranial implants, such as subdural grid electrodes, which may influence CSF leakage, but the total influence of this population on the overall results is expected to be minimal.

Fourth, the results of the risk factor analysis are potentially influenced by confounding. This is inherent to the design of the included publications and the fact that obtained data do not allow correction for potential bias. Future research should further investigate potential risk factors in a multivariate analysis.

Lastly, quality assessment identified only 3 "good quality" studies out of the 26 included in the meta-analysis, compromising quality for the reported outcome measure. The sensitivity analysis shows a higher incidence of CSF leakage in studies of good quality, 7.4 % vs. 4.4% found in all studies which may indicate that the CSF leakage rate in this study may be an underestimation of the true CSF leakage rate.

Despite these limitations this meta-analysis provides a representable overview of the CSF leakage rate and associated risk factors reported in the current body of literature. Moreover, it emphasizes the need for a uniform definition and future studies evaluating CSF leakage and preventative strategies in the pediatric population. CSF leakage may include both incisional leakage and pseudomeningocele (PMC). Incisional CSF leakage is defined as leakage of CSF through the skin, whereas a PMC is an extradural collection of CSF under the skin.[36] Although PMC in the absence of incisional CSF leakage can cause symptoms such as, intracranial hypotension, aseptic meningitis, pain and psychological distress, the condition is often self-limiting .[36, 37] Describing and quantifying symptomatic PMC can be difficult because the diagnosis is subjective in contrast to incisional CSF leakage. Therefore, it should be considered a separate entity.

Conclusions

The overall CSF leakage rate after intradural cranial surgery in the pediatric population is 4.4%. The highest leakage rate is found in patients undergoing a craniectomy. Infratentorial surgery is also associated with higher incidence of CSF leakage, whereas the use of a duraplasty is negatively associated with CSF leak. We emphasize the need for a uniform and clinically meaningful definition of CSF leakage, suggesting "leakage of CSF through the skin".

Funding

EMH Slot receives a research grant through Polyganics b.v., a medical technology company developing a dural sealant. Polyganics b.v. was not involved in the content of this manuscript.

Conflicts of interest/Competing interests

TPC van Doormaal is a consultant for Polyganics b.v.

Polyganics b.v. was not involved in the content of this manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

EMHS, EWH, KvB and TvD contributed to the study conception and design. EMSH and KvB performed the literature search and data collection. NPAZ performed the statistical analyses. The first draft of the manuscript was done by EMHS. All authors critically revised the final manuscript.

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Appendix 1: Pubmed search

("Craniotomy"[Mesh] OR Craniotom*[Title/Abstract] OR Craniectom*[Title/Abstract] OR cranial surgery[Title/Abstract] OR tumor resect*[Title/Abstract] OR tumour resect*[Title/Abstract] OR neurosurgery*[Title/Abstract])

AND

("Cerebrospinal Fluid Leak"[Mesh:NoExp] OR Cerebrospinal Fluid Leak*[Title/Abstract] OR CSF leak*[Title/Abstract] OR pseudomeningocele[Title/Abstract] OR incisional leak*[Title/Abstract] OR "Meningitis"[Mesh] OR meningitis[Title/Abstract] OR "Surgical Wound Infection"[Mesh] OR Surgical Wound Infection*[Title/Abstract] OR wound infection*[Title/Abstract] OR wound leak*[Title/Abstract] OR surgical site infection*[Title/Abstract])

AND

(Infan*[Title/Abstract] OR toddler*[tiab] OR minor[tiab] OR minors*[tiab] OR boy[tiab] OR boys[tiab] OR girl[tiab] OR girls[tiab] OR kid[tiab] OR kids[tiab] OR child[tiab] OR children*[tiab] OR adolescen*[tiab] OR juvenil*[tiab] OR youth*[tiab] OR teen*[tiab] OR pediatrics[MESH] OR pediatri*[tiab] OR paediatri*[tiab] OR youth[tiab] OR youths[tiab] OR teen[tiab] OR teens[tiab] OR teenager[tiab] OR youngster*[tiab] OR child[MeSH])

Appendix 2: Embase search

('craniotomy'/exp OR 'craniotom*':ab,ti OR 'craniectom*':ab,ti OR 'cranial surgery':ab,ti OR 'tumor resect*':ab,ti OR 'tumour resect*':ab,ti OR neurosurgery*:ab,ti)

AND

('liquorrhea'/exp/mj OR 'cerebrospinal fluid leak*':ab,ti OR 'csf leak*':ab,ti OR pseudomeningocele:ab,ti OR 'incisional leak*':ab,ti OR 'meningitis'/exp OR meningitis:ab,ti OR 'surgical infection'/exp OR 'surgical wound infection*':ab,ti OR 'wound infection*':ab,ti OR 'wound leak*:ab,ti' OR 'surgical site infection*':ab,ti)

AND

(infan*:ab,ti OR toddler*:ab,ti OR minor:ab,ti OR minors*:ab,ti OR boy:ab,ti OR boys:ab,ti OR girl:ab,ti OR girls:ab,ti OR kid:ab,ti OR kids:ab,ti OR child:ab,ti OR children*:ab,ti OR adolescen*:ab,ti OR juvenil*:ab,ti OR youth*:ab,ti OR teen*:ab,ti OR 'pediatrics'/exp OR pediatri*:ab,ti OR paediatri*:ab,ti OR youth:ab,ti OR youths:ab,ti OR teen:ab,ti OR teens:ab,ti OR teenager:ab,ti OR youngster*:ab,ti OR 'child'/exp)

AND

[embase]/lim

Appendix 3: Cochrane search

MeSH descriptor: [Craniotomy] explode all trees OR craniotom* OR craniectom* OR cranial surgery OR tumor resect* OR tumour resect* OR neurosurger*

AND

MeSH descriptor: [Cerebrospinal Fluid Leak] explode all trees OR MeSH descriptor: [Meninges] explode all trees OR MeSH descriptor: [Surgical Wound Infection] explode all trees OR Cerebrospinal Fluid Leak OR CSF leak* OR pseudomeningocele OR incisional leak*OR OR meningitis OR Surgical Wound Infection* OR wound infection* OR wound leak*OR surgical site infection*

AND

MeSH descriptor: [Pediatrics] explode all trees OR MeSH descriptor: [Child] explode all trees OR Infan* OR toddler* OR minor OR minors* OR boy OR boys OR girl OR girls OR kid OR kids OR child OR children* OR adolescen* OR juvenil* OR youth* OR teen* OR pediatri* OR paediatri*OR youth OR youths OR teen OR teens OR teenager OR youngster*

Supplementary Information 1.
Overview of quality assessment

Study	Question									Quality rating
	Was the study question or objective clearly stated?	Was the study population clearly and fully described, including a case definition?	Were the cases consecutive?	Were the subjects comparable?	Was the intervention (surgery) clearly described?	Were the outcome measures (CSF leakage) clearly defined, valid, reliable, and implemented consistently across all study participants?	Was the length of follow-up adequate (one month or longer)?	Were the statistical methods well-described (statistical test mentioned)?	Were the results well-described (percentage of CSF leakage or number of CSF leakage mentioned)?	
Cochrane 1994[a]	Yes	Yes	Yes	Yes	No	No	NR	NR	Yes	**Poor**
Bognar 2003	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	**Poor**
Zhou 2014	Yes	Yes	Yes	Yes	No	No	Yes	No	Yes	**Poor**
Culley 1994	Yes	Yes	Yes	Yes	No	No	NR	Yes	Yes	**Poor**
Gopalakrishnan 2012	Yes	Yes	Yes	Yes	No	No	NR	Yes	Yes	**Poor**
Houdemont 2011	Yes	Yes	Yes	Yes	No	No	NR	Yes	Yes	**Poor**
Parizek 1998	Yes	Yes	Yes	Yes	Yes	No	NR	NR	Yes	**Poor**
Srinivasan 1999	No	Yes	Yes	Yes	Yes	No	Yes	NA	Yes	**Poor**
Panigrahi 2012	Yes	Yes	CD	Yes	Yes	No	NR	NA	Yes	**Poor**
Hale 2019	Yes	Yes	Yes	Yes	No	No	NR	Yes	Yes	**Poor**
Krieger 1999	Yes	Yes	Yes	Yes	Yes	No	Yes	NR	Yes	**Fair**
Dlouhy 2015	Yes	Yes	Yes	Yes	Yes	No	Yes	NR	Yes	**Fair**
Hidalgo 2018	Yes	Yes	Yes	Yes	Yes	No	Yes	NR	Yes	**Fair**
Vedantam 2017	Yes	Yes	CD	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Gnanaligham 2003[a]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Gnanaligham 2002	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Kushel 2019	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Levy 2003	Yes	Yes	Yes	Yes	Yes	No	Yes	NR	Yes	**Fair**
Parker 2011	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Soleman 2019[a]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Jiang 2018[b]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Roth 2018	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	**Fair**
Lassen 2011[a]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	**Good**
Muszynski 1994	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	**Good**
Hosainey 2014	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	**Good**
Steinbok 2007	Yes	Yes	Yes	Yes	Yes	Yes	NR	Yes	Yes	**Good**

Legend	
[a]	not included in meta-analysis because of overlap with another study included in the meta-analysis.
[b]	not included in meta-analysis because of overestimation of CSF leakage because overestimation of CSF leakage resulting from wound drainage and inclusion of clear production in the drainage system as CSF leakage
CD	Cannot determine
NA	Not applicable
NR	Not reported
Yes	Low risk of bias
CD/NA/NR	Unclear risk of bias
No	High risk of bias

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Incisional CSF leakage after intradural cranial surgery in children: incidence, risk factors, and complications

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Journal of Neurosurgery: Pediatrics. 2023;3:1-8. doi: 10.3171/2022.11.PEDS22421. Epub ahead of print.

Abstract

Objective

The risk of CSF leakage after cranial surgery and its associated complications in children are unclear because of variable definitions and the lack of multicenter studies. In this study the authors aimed to establish the incidence of CSF leakage after intradural cranial surgery in the pediatric population. In addition, we evaluated potential risk factors and complications related to CSF leakage in the pediatric population.

Methods

The authors performed an international multicenter retrospective cohort study in three tertiary neurosurgical referral centers. All patients were included who were aged 18 years or younger and had undergone cranial surgery to reach the subdural space during the period between 2015 and 2021. Patients who died or were lost to follow-up within 6 weeks after surgery were excluded. The primary outcome measure was the incidence of CSF leakage, defined as leakage through the skin, within 6 weeks after surgery. Univariable and multivariable logistic regression analyses were performed to identify risk factors for and complications related to CSF leakage.

Results

In total, 759 procedures were identified, performed in 687 individual patients. The incidence of CSF leakage was 7.5% (95% CI 5.7–9.6). In the multivariate model, independent risk factors for CSF leakage were hydrocephalus (OR 4.5, 95% CI 2.2–8.9) and craniectomy (OR 7.6, 95% CI 3.0–19.5). Patients with CSF leakage had higher odds of pseudomeningocele (5.7, 95% CI 3.0–10.8), meningitis (21.1, 95% CI 9.5–46.8), and surgical site infection (7.4, 95% CI 2.6–20.8) than patients without leakage.

Conclusions

CSF leakage risk in children after cranial surgery, which is comparable to the risk reported in adults, is an event of major concern and has a serious clinical impact.

Keywords

cerebrospinal fluid leakage; pediatrics; infection; cranial surgery; hydrocephalus; craniectomy

Abbreviations

aOR = adjusted OR; ELD = external lumbar drainage; ETV = endoscopic third ventriculostomy; EVD = external ventricular drainage; PICU = pediatric intensive care unit; PMC = pseudomeningocele; SSI = surgical site infection; VIF = variance of inflation factor; VP = ventriculoperitoneal.

Introduction

Cerebrospinal fluid (CSF) leakage is a well-known complication of neurosurgery that may lead to wound-healing problems, pneumocephalus, surgical site infection (SSI), and meningitis. In addition to increased morbidity, CSF leakage has been associated with increased healthcare cost[1,2].

We performed a systematic review and meta-analysis and concluded that definitions of CSF leakage are highly variable and that instances are underreported[3]. This lack of consistency has resulted in a wide interval of presumed risk of CSF leakage after neurosurgery in children (0%–38%), with an average risk of 4.4%[3]. Thereby extensive research into risk factors of CSF leakage after intradural cranial surgery in the pediatric population is lacking, and the majority of studies only report on specific subcategories, for example, posterior fossa tumor surgery[4-7].

The term CSF leakage may be used imprecisely to mean both incisional leakage and pseudomeningocele (PMC). Incisional CSF leakage is defined as leakage of CSF through the skin, whereas PMC is a subcutaneous collection of CSF[8,9]. Although PMC without incisional CSF leakage may lead to intracranial hypotension, aseptic meningitis, pain, and psychological distress, it is often self-limiting[9].Therefore, in the present study CSF leakage was defined as incisional CSF leakage and PMC was considered a separate entity.

In this study we aimed to establish the incidence of incisional CSF leakage after intradural cranial surgery in the pediatric population within 6 weeks after surgery. In addition, we sought to evaluate the risk factors and complications associated with CSF leakage in the pediatric population. Establishing the clinically meaningful impact of CSF leakage in children and identifying risk factors will give further direction to the development of strategies to prevent CSF leakage.

Methods

Study Design

This multicenter historical cohort study included pediatric patients who were consecutively operated on between January 1, 2015, and June 30, 2021, in the Wilhelmina Children's Hospital and Princess Máxima Center for Pediatric Oncology in Utrecht, the Netherlands, and the University Children's Hospital and the University Hospital in Zurich, Switzerland. This study was approved by the institutional review boards and the local ethics committees and conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[10].

Study Population

All patients aged 18 years or younger who underwent intradural cranial surgery and for whom a complete surgical report was available were considered for inclusion. We excluded data for patients who had undergone burr-hole surgery (i.e., drainage of chronic subdural hematoma, ventriculoperitoneal [VP] shunt placement, or endoscopic third ventriculostomy [ETV]) or transsphenoidal surgery and patients who had died within 6 weeks after surgery or were lost to follow-up. If reoperation occurred within 6 weeks for reasons other than treatment of CSF leakage, the case was excluded. A 6-week period was chosen to reflect a realistic follow-up duration for postoperative neurosurgical cases, taking into consideration that postoperative CSF leakage outside this window is unlikely. Inclusion and exclusion criteria are presented in **Supplemental Table 1**.

Data Collection

Procedures eligible for inclusion were selected based on operation codes in the electronic patient records or a prospectively collected database. Data were collected by screening of medical notes, medical imaging, and surgical reports in the electronic patient record from 6 weeks prior to surgery to 6 weeks after surgery for all eligible patients.

Collected patient data included age at time of surgery, steroid use for at least 2 consecutive days directly before or after surgery, diagnosis of hydrocephalus based on medical imaging or medical notes, perioperative CSF diversion surgery (external ventricular drainage [EVD], external lumbar drainage [ELD], and VP shunt or ETV), perioperative chemotherapy, and perioperative radiotherapy. Patients treated with steroids immediately before or after surgery or with perioperative chemotherapy (within 6 weeks before or after surgery), and patients with known autoimmune disease were classified as immunocompromised. Surgical characteristics included neurosurgical center, anatomical location of the durotomy (infratentorial or supratentorial), indication for surgery (tumor, vascular, Chiari decompression, epilepsy, trauma, infection), reoperation (defined as any consecutive surgery at the same anatomical location), use of products with the intention to seal the dura, use of duraplasty, emergency surgery, replacement of the bone flap (craniotomy/craniectomy), and watertight closure attempt (yes/no).

Characteristics with respect to the clinical course included total number of admission days, number of days in the pediatric intensive care unit (PICU), and new neurological deficit after surgery (including worsening of existing deficit). In case of CSF leakage the following additional data were collected: the estimated number of extra admission days due to CSF leakage (estimated by subtracting the standard number of admission days necessary for the procedure from the actual days in hospital), surgical wound revision because of CSF leakage, CSF-diverting procedure because of CSF leakage, number of

days of CSF diversion, lumbar puncture to treat CSF leakage, extra suture placement or pressure bandage because of CSF leakage, and puncture of PMC.

The primary outcome measure was CSF leakage within 6 weeks after surgery, defined as leakage of CSF through the skin (either confirmed with beta-2-transferrin test or reported in the clinical notes). Secondary outcome measures included the presence of PMC (defined as a subcutaneous collection of CSF), meningitis (suspicion or diagnosis of meningitis based on antibiotic treatment according to the clinical notes or positive CSF culture) and SSI (diagnosis or suspicion of SSI based on antibiotic treatment according to the clinical notes or positive wound culture) within 6 weeks after surgery.

Statistical Analyses

Baseline characteristics were compared between patients with and those without CSF leakage by using the Mann-Whitney U-test for nonnormally distributed continuous variables and a chi-square test (or Fishers exact test for contingency tables with cells below 5) for categorical variables. The primary outcome measure is presented as percentage of the total population. Univariable logistic regression was used to determine the odds ratios (ORs) for potential risk factors for CSF leakage and secondary complications in relation to CSF leakage. Multivariable logistic regression analysis was subsequently applied to identify risk factors for CSF leakage, including variables of the univariable regression with a significance level of $p < 0.05$ limited to 1 variable per 10 observations of the primary outcome (in order of significance level)[11].Risk factors are presented as the OR with 95% confidence interval (CI). The same statistical analysis was applied to evaluate the adjusted ORs for secondary complications in relation to CSF leakage. Multicollinearity between all variables in the multivariable model was assessed using the variance of inflation factor (VIF), and p values < 0.05 were considered statistically significant. All analyses were performed in IBM SPSS version 26.0 (IBM Corp.).

Results

Demographics

We included a total of 759 intradural cranial procedures in the study data, representing 687 individual patients **(Figure 1)**. The most common indication for surgery was tumor resection (44.8%). The location of the durotomy was infratentorial in 23.8% of procedures **(Table 1)**. A total of 36 procedures (4.7%) included a craniectomy (Chiari decompression, n = 15 [41.7%]; vascular, n = 7 [19.4%]; trauma, n = 6 [16.7%]; tumor, n = 5 [13.9%]; infection, n = 3 [8.3%]), among which 41.7% were emergency procedures. In our cohort, craniectomies comprised 11.0% of infratentorial surgeries and 2.8% of supratentorial procedures. Hydrocephalus was present in 45.3% of infratentorial

versus 8.1% of supratentorial procedures. Compared to patients without CSF leak, significantly more patients with CSF leak had CSF diversion ($p < 0.001$) **(Table 1)**, and the percentage of patients who had undergone CSF diversion prior to the index procedure was significantly higher in patients with CSF leakage (31.6 vs 10.0%, $p < 0.001$) **(Supplemental Table 2)**. Similarly, placement of CSF diversion during the index procedure occurred more often in patients with CSF leakage (5.3% vs 1.0%, $p= 0.033$). After the index procedure, a CSF diversion procedure for reasons other than CSF leakage was performed in 24 patients, of whom 11 patients had already undergone such a procedure prior to or during the index procedure. There were significantly more patients with CSF leakage who underwent CSF diversion postprocedure than patients without CSF leakage (17.5% vs 2.0%, $p < 0.001$).

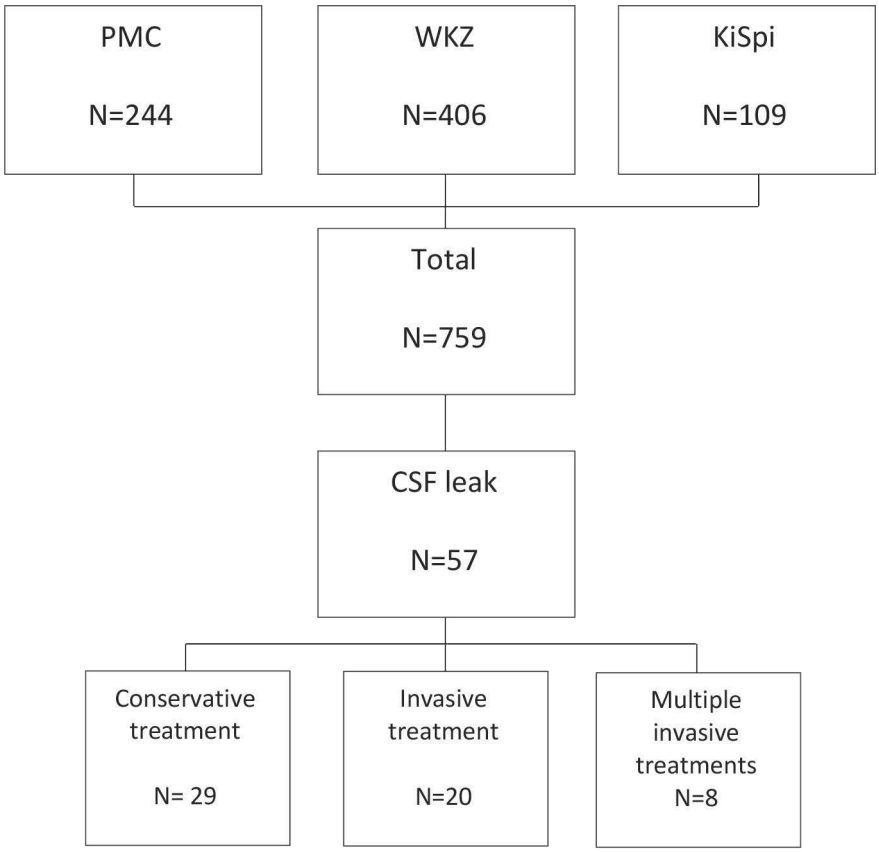

Figure 1. Flowchart of the study population.

KiSpi = University Children's Hospital Zurich; PMC = Princess Máxima Center for Pediatric Oncology; WKZ = Wilhelmina Children's Hospital.

Table 1. Patient and treatment characteristics

Variable	Total (n = 759)	CSF Leakage		p Value
		Yes (n = 57)	No (n = 702)	
Patient characteristics				
Age, yrs	9 (4–13)	6 (2–12)	9 (4–13)	0.059[a]
Female sex	355 (46.8)	21 (36.8)	334 (47.6)	0.118[b]
Craniectomy	36 (4.7)	10 (17.5)	26 (3.7)	<0.001*[c]
Infratentorial op	181 (23.8)	25 (43.9)	156 (22.2)	<0.001*[b]
Immunocompromised	233 (30.7)	29 (50.9)	204 (29.1)	0.001*[b]
Radiation therapy	89 (11.7)	13 (22.8)	76 (10.8)	0.007*[b]
Chemotherapy	108 (14.2)	15 (26.3)	93 (13.2)	0.007*[b]
Hydrocephalus	129 (17.0)	26 (45.6)	103 (14.7)	<0.001*[b]
CSF diversion	111 (14.6)	25 (43.9)	86 (12.3)	<0.001*[b]
Op characteristics				
Neurosurgical center				0.471[b]
Princess Maxima Center for pediatric oncology	244 (32.1)	22 (9.0)	222 (91.0)	
Wilhelmina Children's Hospital	406 (53.5)	29 (7.1)	377 (92.9)	
University Children's Hospital Zurich	109 (14.4)	6 (5.5)	103 (94.5)	
Reop	161 (21.2)	15 (26.3)	146 (20.8)	0.327[b]
Emergency op	56 (7.4)	10 (17.5)	46 (6.6)	0.006[c]
Indication				0.022*[c]
Tumor	340 (44.8)	33 (57.9)	307 (43.7)	
Vascular	63 (8.3)	3 (5.3)	60 (8.5)	
Chiari	15 (2.0)	2 (3.5)	13 (1.9)	
Epilepsy	324 (42.7)	16 (28.1)	308 (43.9)	
Trauma	6 (0.8)	2 (3.5)	4 (0.6)	
Infection	11 (1.4)	1 (1.8)	10 (1.4)	
Watertight closure	592 (92.1)	41 (89.1)	551 (92.3)	0.399[c]
Duraplasty use	195 (28.6)	19 (35.2)	176 (28.0)	0.264[b]
Sealant use	327 (47.5)	24 (44.4)	303 (47.8)	0.636[b]
Clinical course				
Days in hospital	6 (5–9)	16 (9–22.5)	6 (5–8)	<0.001*[a]
Days in PICU	1 (1–1)	1 (1–2.5)	1 (1–1)	<0.001*[a]
New/increased neurological deficit	160 (21.1)	27 (47.4)	133 (18.9)	<0.001*[b]

All values are reported as number (%) for categorical variables or median (IQR) for nonnormally distributed continuous variables. Percentage of data missing: watertight closure 15.2%, duraplasty use 10.1%, sealant use 9.4%, and number of days in hospital 0.1%. No data were missing for the remaining variables.
* Significant at $p < 0.05$; [a]Mann-Whitney U-test, [b] chi-square test, [c] Fishers exact test.

Incidence of CSF Leakage

CSF leakage within 6 weeks after surgery occurred in 57 of 759 (7.5%, 95% CI 5.7%–9.6%) surgical procedures. There was no association between neurosurgical center of admission and CSF leakage (p = 0.471) **(Table 1)**.

The incidences of CSF leakage were 13.8% in infratentorial procedures and 5.5% in supratentorial procedures, and 27.8% in craniectomies and 6.5% in craniotomies. CSF leakage incidence was highest in trauma procedures (33.3%) and lowest in vascular surgery (4.8%) **(Supplemental Table 3)**.

Risk Factor Analysis

Univariable analyses revealed evidence for strong associations between CSF leakage and craniectomy, infratentorial surgery, emergency surgery, compromised immunity, perioperative radiation therapy, chemotherapy, and hydrocephalus. Furthermore, univariable analysis of indications showed a significant association between CSF leakage and epilepsy surgery **(Table 2)**.

Table 2. Univariable logistic regression analysis for CSF leakage.

Variable	OR	95% CI		p Value
		Lower Limit	Upper Limit	
Age	1.0	0.9	1.0	0.058
Male sex	1.6	0.9	2.7	0.121
Craniectomy	5.5	2.5	12.2	<0.001*
Infratentorial	2.7	1.6	4.8	<0.001*
Reop	1.4	0.7	2.5	0.329
Emergency op	3.0	1.4	6.4	0.004*
Indication				
Tumor†				0.053
Vascular	0.5	0.1	1.6	0.217
Chiari	1.4	0.3	6.6	0.646
Epilepsy	0.5	0.3	0.9	0.021*
Trauma	4.7	0.8	26.4	0.082
Infection	0.9	0.1	7.5	0.946
Sealant use	0.9	0.5	1.5	0.637
Duraplasty use	1.4	0.8	2.5	0.266
Watertight dural closure	0.7	0.3	1.8	0.447
Immunocompromised	2.5	1.5	4.4	0.001*
Radiation therapy	2.4	1.3	4.7	0.009*
Chemotherapy	2.3	1.3	4.4	0.008*
Hydrocephalus	4.9	2.8	8.5	<0.001*

* Significant at p < 0.05.
† Reference category.

After multivariable analysis, only hydrocephalus (adjusted OR [aOR] 4.5, 95% CI 2.2–8.9) and craniectomy (aOR 7.6, 95% CI 3.0–19.5) showed strong evidence for an independent association with CSF leakage **(Table 3)**.

The multivariable analysis revealed no indication of multicollinearity among predictor variables (VIF 1.163–1.727).

Table 3. Multivariable logistic regression for CSF leakage

Variable	aOR	95% CI		p Value	VIF
		Lower Limit	Upper Limit		
Craniectomy	7.6	3.0	19.5	<0.001*	1.170
Infratentorial	1.1	0.5	2.1	0.888	1.347
Emergency op	1.3	0.6	3.0	0.561	1.163
Immunocompromised	1.5	0.7	3.1	0.329	1.727
Chemotherapy	1.7	0.7	3.8	0.214	1.509
Hydrocephalus	4.5	2.2	8.9	<0.001*	1.316

* Significant at $p < 0.05$.

Complications Related to CSF Leakage

Pseudomeningocele occurred in 11.3% of procedures. CSF leakage was accompanied by PMC in 42.1% of cases, compared to 8.8% when no CSF leakage was present ($p < 0.001$). Overall incidences of 4.1% for meningitis and 2.2% for SSI were observed in our study population. Both meningitis and SSI occurred significantly more frequently in patients with than those without CSF leakage (31.6% vs 1.9%, $p < 0.001$, and 10.5% vs 1.6%, $p = 0.001$). In patients with compared to patients without CSF leakage, the SSI incidence OR was 7.4 (95% CI 2.6–20.8) **(Table 4)**. Univariable analysis did not identify significant associations between age at surgery, sex, neurosurgical center, indication, sealant use, duraplasty use, perioperative radiation therapy, perioperative chemotherapy, reoperation, immunocompromised status, or SSI **(Supplemental Table 4)**. For meningitis incidence in patients with CSF leakage compared to those without the OR was 24.5 (95% CI 11.2–53.5) **(Table 4)**. In univariable analyses, meningitis was associated with epilepsy surgery, chemotherapy, immunocompromised status, and age at surgery **(Supplemental Table 5)**. After correction for chemotherapy and immunocompromised status in multivariable analysis of meningitis in patients with CSF leakage compared to those without, the OR was 21.1 (95% CI 9.5–46.8) **(Table 5)**.

Table 4. Complications associated with CSF leakage

Variable	CSF Leakage		OR	95% CI		p Value
	Yes (n = 57)	No (n = 702)		Lower Limit	Upper Limit	
PMC	24 (42.1)	62 (8.8)	7.5	4.2	13.5	<0.001*
Meningitis	18 (31.6)	13 (1.9)	24.5	11.2	53.5	<0.001*
SSI	6 (10.5)	11 (1.6)	7.4	2.6	20.8	<0.001*

* Significant at $p < 0.05$.

Table 5. Multivariable variable logistic regression for meningitis

Variable	aOR	95% CI		p Value
		Lower Limit	Upper Limit	
Chemotherapy	1.4	0.5	4.1	0.524
Immunocompromised	2.0	0.7	5.2	0.173
CSF leakage	21.1	9.5	46.8	<0.001*

* Significant at $p < 0.05$.

The OR for PMC in patients with CSF leakage compared to those without was 7.5 (95% CI 4.2–13.5) **(Table 4)**. Univariable analyses showed significant associations between PMC and emergency surgery, male sex, hydrocephalus, age at surgery, and craniectomy **(Supplemental Table 6)**. Including these variables in multivariable analysis resulted in an adjusted OR of 5.7 (95% CI 3.0–10.8) for patients with CSF leakage compared to those without **(Table 6)**.

Table 6. Multivariable variable logistic regression for PMC

Variable	aOR	95% CI		p Value
		Lower Limit	Upper Limit	
Age	0.9	0.9	1.0	0.014*
Craniectomy	2.5	1.0	6.3	0.043*
Hydrocephalus	1.2	0.7	2.2	0.560
Emergency op	1.2	0.5	2.7	0.678
Male sex	1.8	1.1	2.9	0.022*
CSF leakage	5.7	3.0	10.8	<0.001*

* Significant at $p < 0.05$.

Treatment of CSF Leakage

A median estimated 3 (IQR 0–11) days of prolonged hospital stay contributed to CSF leakage. A total of 13 of 57 (22.8%) patients were readmitted because of CSF leakage. Invasive treatment (defined as a CSF-diverting procedure, lumbar puncture, puncture of the PMC, or surgical wound revision) was performed in 49.1% of procedures **(Table 7)**. Invasive treatment of the PMC was performed in 20.8% of procedures if it was accompanied by CSF leakage, whereas the PMC was punctured in 3.2% of procedures in which there was no CSF leakage (p = 0.017). A surgical wound revision was performed in 17.5% of procedures **(Table 7)**. In 3 of 10 patients wound revision was performed after initial treatment with a CSF-diverting procedure. In 1 patient a CSF-diverting procedure was performed after revision surgery. CSF leakage occurred at a median of 7 days median (IQR 4–10). The median time between a CSF-diverting procedure and diagnosis of CSF leakage was 1 day (IQR 1–3) and for wound revision was 2 (IQR 0.75–12.75). CSF-diverting drains were kept in place for a median of 6 (IQR 5–8) days on average.

Table 7. Treatment of 57 patients with CSF leakage

	No. (%)
Patients with CSF leakage	57 (100)
Invasive treatment	28 (49.1)
Surgical wound revision	10 (17.5)
CSF diverting procedure	21 (36.8)
EVD	7 (12.3)
VPS	2 (3.5)
ELD	12 (21.1)
Lumbar puncture	0 (0.0)
Puncture of PMC	5 (8.8)
Multiple invasive treatments	8 (14.0)
Conservative treatment	36 (63.2)
Pressure bandage	30 (52.6)
Additional sutures	19 (33.3)

A total of 29 patients did not undergo invasive treatment, and of these 21 patients received additional sutures and/or a pressure bandage. There were 8 patients who did not receive treatment specifically for CSF leakage; 5 of these patients already had an EVD in place for hydrocephalus treatment.

Eleven of 18 (61.1%) patients in whom meningitis occurred underwent wound revision or CSF diversion for CSF leakage. There was no significant difference in the time between

diagnosis of CSF leak and first surgical treatment (wound revision or CSF diversion) of this CSF leak in patients with compared to patients without meningitis (median time 2 [IQR 1–3] vs 1 [1–2.75] days, p = 0.251).

Discussion

According to the findings of this study, the incidence of CSF leakage in the pediatric population is 7.5% (95% CI 5.7–9.6).

This study is, to our knowledge, the first CSF leakage investigation performed in a multicenter setting to define the occurrence of this complication with a prespecified definition as, "leakage of CSF through the skin," within the entire spectrum of pediatric intradural cranial neurosurgery procedures, instead of as a separate subgroup based on a particular indication or location (e.g., posterior fossa tumor surgery). Therefore, we believe the study results are robust and generalizable.

The CSF leakage incidence found in the current study is higher than that found in our recent meta-analysis (4.4%, 95% CI 2.6–7.3%)[3]. This result is likely caused by the high portion of case series of low to fair quality in which CSF leakage incidence was not the primary outcome measure and was often not specifically defined. The incidence found in the current study is similar to that observed in the sensitivity analysis of the meta-analysis including only high-quality studies (7.4%; 95% CI 4.6–11.6)[3].The incidence of CSF leakage in the current pediatric population is also comparable to that found in a recent study in the adult population (7.5%)[12].

Risk factors for CSF leakage identified in this study are hydrocephalus (OR 4.5, 95% CI 2.2–8.9) and craniectomy (OR 7.6, 95% CI 3.0–19.5). This result is in accordance with findings in previous publications[3-7]. The CSF leakage rate in our subgroup of craniectomy procedures is comparable to that found by Gnanalingham et al. (27%)[6]. The increased CSF leak risk may be explained by the lack of rigid support, otherwise provided by the replaced bone flap, which allows the dura to bulge outward, combined with pulsatile CSF dynamics[6].Preventative strategies should aim at adequately controlling hydrocephalus and avoiding craniectomy.

Infratentorial surgery has been reported as a risk factor for CSF leakage in previous studies, yet was not significantly associated in our multivariate analysis[3-5, 12].This suggests that factors relating to CSF pressure dynamics are most important in predicting CSF leakage, and thus adequate control of CSF flow should be sought in order to prevent it[5,6].Younger age and male sex have also been reported by some studies as risk factors for CSF leakage, which was not replicated by the current study[4, 5, 12].

Although meticulous watertight closure of the dura is commonly accepted to be of utmost importance for the prevention of CSF leakage, especially in posterior fossa surgery, we did not find a significant protective effect in this study. This result is in line with results of a previous study from Roth et al.[13].The analysis in the current study, however, was limited by the relatively large proportion of missing values, and a small number of procedures in watertight dura closure were not performed.

Furthermore, we did not find a significant association for duraplasty use or sealant use and CSF leakage. These findings are contrary to those for recent study by Hale et al.[7]; however, their study included posterior fossa tumor resection specifically. Sealants have been reported to be protective against CSF leakage, although evidence is inconsistent and currently no dural sealants have been prospectively investigated in children[1, 14-18].

The incidence of PMCs found in this study (11.3%) is higher than that reported by Norrdahl et al.[19] (5.1%). This discrepancy may be due to the fact that in the previous study PMCs that occurred after admission were captured only if they required readmission, because postoperative visits were not conducted in the same facility, thus leading to underestimation of the true incidence, because most PMCs are self-limiting[9].

The overall incidence of meningitis in our population (4.1%) is on the lower end of the range reported for other studies (2%–15%)[5, 6, 20-22].Yet, the meningitis risk found in patients with CSF leakage (31.6%) is on the upper range compared to previous studies (10%–35%)[5, 6, 22], further emphasizing the association between CSF leakage and infection. A limitation of the analysis in this study is that our definition of meningitis does not require a positive CSF culture, which may have contributed to a slight overestimation of the infectious meningitis incidence, which is also reflected by a strong association between SSIs and CSF leakage found in our study, in which the overall incidence of SSI was relatively low compared to the incidence in previous cohorts of cranial surgery patients (0%–7.5%)[5-7, 13].

In 49.1% of patients an invasive treatment (defined as a CSF-diverting procedure, lumbar puncture, puncture of the PMC, or surgical wound revision) was required for CSF leakage. The invasive treatment and prolonged hospitalization necessitated by CSF leakage constitutes a substantial treatment burden, especially in the pediatric population. Lassen et al.[5] report 70% invasive treatment in their series, with no cases of revision surgery. The CSF diversion procedures reported in their study, however, also included those for treatment of hydrocephalus in procedures in which conservative treatment resolved the CSF leakage itself. Wound revision surgery was performed in 17.5% of procedures in our series compared to 31% in the adult population[12].The positive experiences in our centers with conservative treatment among the dedicated pediatric neurosurgeons may have made them more inclined to adhere to a conservative strategy,

which is also reflected by the time between diagnosis of CSF leakage and invasive treatment. The relatively high proportion of children successfully treated for CSF leakage conservatively indicates that initial conservative treatment is a viable option.

This study is subject to a number of limitations. Although to our knowledge this study included the largest analysis to date of CSF leakage in pediatric patients, the frequency of the primary outcome was still low. Therefore, the multivariable analysis included only variables that were significant in the univariable analysis, with a limit of 6 variables, and thus did not include indications for surgery and radiation therapy. This limitation may have led to over- or underestimation of the effect sizes observed in the multivariate analysis, which must thus be interpreted with caution[23]. Furthermore, this study may be subject to selection bias as patients who died within 6 weeks after surgery were excluded, which may have influenced the CSF incidence, because patients at higher risk of death after surgery (patients with trauma or infectious complications) may also have been at higher risk for CSF leakage. Finally, this study relies on retrospective data and thus is subject to reporting bias and observer bias. Underreporting of CSF leakage that resolved with pressure bandage or additional suture placement may have caused a slight underestimation of the CSF leakage incidence found in this study. Similarly, the use of sealants or duraplasty material and the intent of watertight closure was not always registered, which may have led to underestimation of these variables and their effects.

Conclusions

The risk of CSF leakage risk in children undergoing cranial surgery is significant (7.5%), comparable to the risk reported in adults, and has a serious clinical impact. Hydrocephalus and craniectomy are potential risk factors for CSF leakage, after correction for infratentorial surgery, emergency surgery, immunocompromised status, and chemotherapy. Future studies should investigate preventative strategies specifically for the pediatric population.

Disclosures

Dr. Slot and Dr. van Doormaal reported grants from Polyganics B.V., a biotechnology company developing a dural sealant, during the conduct of the study and Dr. Regli reported grants from Polyganics B.V. outside the submitted work.

Author Contributions

Conception and design: Slot, van Doormaal, Hoving. Acquisition of data: Slot, Krayenbühl, Regli, Germans. Analysis and interpretation of data: Slot, van Doormaal, van Baarsen. Drafting the article: Slot, van Doormaal. Critically revising the article: all authors. Reviewed submitted version of manuscript: van Doormaal, van Baarsen, Krayenbühl, Germans, Hoving. Approved the final version of the manuscript on behalf of all authors: Slot. Statistical analysis: Slot. Administrative/technical/material support: Slot, Regli. Study supervision: van Doormaal, Regli, Hoving.

Supplemental Information

Online-Only Content

Previous Presentations

Preliminary results of this study were shared in a poster presentation at the European Association of Neurosurgical Societies (EANS) Conference, Belgrade, Serbia, October 16–20, 2022, and the International Society for Pediatric Neurosurgery Meeting, Singapore, December 6–10, 2022.

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Supplemental table 1. Inclusion and exclusion criteria

Inclusion criteria
Intradural cranial surgery
≤18 years old at time of surgery
Surgical report available
Exclusion criteria
Transsphenoidal surgery
Burr-hole surgery
Death within 6 weeks after surgery
Lost to follow-up within 6 weeks after surgery
Reoperation for other reason than CSF leakage within 6 weeks after surgery

Supplemental table 2. CSF diversion and timing.

	Total	**CSF leak**	**No CSF leak**	**P-value**
Preoperative CSF diversion	88 (11.6)	18 (31.6)	70 (10.0)	<0.001*[a]
EVD	40 (5.3)	13 (22.8)	27 (3.8)	
VPS	31 (4.1)	1 (1.8)	30 (4.3)	
ELD	1 (0.1)	0 (0.0)	1 (0.1)	
ETV	16 (2.1)	4 (7.0)	12 (1.7)	
Intraoperative CSF diversion	10 (1.3)	3 (5.3)	7 (1.0)	0.033*[a]
EVD	9 (1.2)	3 (5.3)	6 (0.9)	
VPS	1 (0.1)	0 (0.0)	1 (0.1)	
Postoperative CSF diversion	24 (3.2)	10 (17.5)	14 (2.0)	<0.001*[a]
EVD	5 (0.7)	2 (3.5)	3 (0.4)	
VPS	13 (1.7)	4 (7.0)	9 (1.3)	
ELD	1 (0.1)	0 (0.0)	1 (0.1)	
ETV	5 (0.7)	4 (7.0)	1 (0.1)	

[a]Fisher's Exact test
CSF: cerebrospinal fluid
ELD: external lumbar drain
ETV: endoscopic third ventriculostomy
EVD: external ventricular drain
VPS: ventriculoperitoneal shunt
*Significant

Supplemental table 3. CSF leakage by indication.

Indication	N (759)	CSF leakage, N (%)
Tumor	340	33 (9.7)
Epilepsy	324	16 (4.9)
Vascular	63	3 (4.8)
Chiari	15	2 (13.3)
Infection	11	1 (9.1)
Trauma	6	2 (33.3)

The CSF leakage incidence is reported as number with percentage within the subgroup based on indication between brackets.
CSF: cerebrospinal fluid.

Supplemental table 4. Univariable logistic regression for surgical site infection.

Variable	OR	Lower limit 95% CI	Upper limit 95% CI	P-value
Age	1.0	0.9	1.1	0.877
Sex (male)	1.3	0.5	3.4	0.641
Reoperation	0.8	0.2	2.8	0.717
Neurosurgical center				
1[a]				0.451
2	2.3	0.6	8.1	0.220
3	2.3	0.5	11.4	0.319
Indication				
Tumor[a]				0.991
Vascular	0.8	0.1	6.3	0.806
Chiari	0.0	0.0		0.999
Epilepsy	1.4	0.5	3.7	0.547
Trauma	0.0	0.0		0.999
Infection	0.0	0.0		0.999
Sealant use	1.6	0.6	4.2	0.349
Duraplasty use	0.5	0.2	1.9	0.320
Immunocompromised	1.6	0.6	4.3	0.348
Radiation therapy	0.5	0.1	3.5	0.460
Chemotherapy	0.8	0.2	3.5	0.769
CSF leakage	7.4	2.6	20.8	<0.001*

The odds ratio (OR) and lower and upper limit of the 95% confidence intervals (CI) are reported. P-values <0.05 are considered significant.
[a] Reference category
*Significant
CSF: cerebrospinal fluid

Supplemental table 5. Univariable logistic regression for meningitis.

Variable	OR	Lower limit 95% CI	Upper limit 95% CI	P-value
Age	0.9	0.8	1.0	0.010*
Sex (male)	1.1	0.5	2.2	0.854
Reoperation	0.7	0.3	1.9	0.482
Neurosurgical center				
1[a]				0.223
2	0.5	0.2	1.1	0.085
3	0.8	0.3	2.3	0.659
Indication				
Tumor[a]				0.151
Vascular	0.0	0.0		0.997
Chiari	1.1	0.1	8.7	0.939
Epilepsy	0.3	0.1	0.8	0.014*
Trauma	3.0	0.3	27.2	0.320
Infection	1.5	0.2	12.4	0.697
Sealant use	1.3	0.6	2.7	0.516
Duraplasty use	0.9	0.4	2.1	0.811
Watertight dural closure	0.5	0.2	1.5	0.212
Immunocompromised	3.3	1.6	6.9	0.001*
Radiation therapy	2.3	1.0	5.5	0.062
Chemotherapy	3.1	1.4	6.7	0.005*
CSF leakage	24.5	11.2	53.5	<0.001*

The odds ratio (OR) and lower and upper limit of the 95% confidence intervals (CI) are reported. P-values <0.05 are considered significant.
[a] Reference category
*Significant
CSF: cerebrospinal fluid

Supplemental table 6. Univariable logistic regression for pseudomeningocele.

Variable	OR	Lower limit 95% CI	Upper limit 95% CI	P-value
Age	0.9	0.9	1.0	0.003*
Sex (male)	2.0	1.2	3.2	0.006*
Craniectomy	3.8	1.8	8.0	<0.001*
Infratentorial	0.8	0.4	1.3	0.347
Reoperation	0.7	0.4	1.3	0.237
Emergency surgery	2.0	1.0	4.1	0.045*
Neurosurgical center				
1[a]				0.671
2	1.3	0.8	2.1	0.383
3	1.2	0.6	2.5	0.554
Indication				
Tumor[a]				0.264
Vascular	1.0	0.4	2.4	0.964
Chiari	1.4	0.3	6.6	0.646
Epilepsy	1.3	0.8	2.1	0.278
Trauma	4.7	0.8	26.4	0.082
Infection	3.5	0.9	13.8	0.075
Sealant use	0.8	0.5	1.2	0.242
Duraplasty use	1.1	0.7	1.9	0.630
Watertight dural closure	1.9	0.6	6.4	0.277
Immunocompromised	1.4	0.9	2.2	0.166
Radiation therapy	1.1	0.6	2.2	0.754
Chemotherapy	0.9	0.5	1.7	0.685
Hydrocephalus	1.8	1.1	3.1	0.026*
CSF leakage	7.5	4.2	13.5	<0.001*

The odds ratio (OR) and lower and upper limit of the 95% confidence intervals (CI) are reported. P-values <0.05 are considered significant.
[a] Reference category
*Significant
CSF: cerebrospinal fluid

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Cerebrospinal fluid leakage after intradural spinal surgery in children

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Child's Nervous System. 2023. doi: 10.1007/s00381-022-05797-w. Epub ahead of print.

Abstract

Purpose

This study aimed to establish the incidence of CSF leakage in children and associated complications after intradural spinal surgery in three tertiary neurosurgical referral centers and to describe the treatment strategies applied.

Methods

Patients of 18 years or younger who underwent intradural spinal surgery between 2015 and 2021 in three tertiary neurosurgical referral centers were included. Patients who died or were lost to follow-up within six weeks after surgery were excluded. The primary outcome measure was CSF leakage within six weeks after surgery, defined as leakage of CSF through the skin. Secondary outcome measures included: presence of pseudomeningocele (PMC), meningitis and surgical site infection (SSI).

Results

We included a total of 75 procedures, representing 66 individual patients. The median age in this cohort was 5 (IQR 0-13) years. CSF leakage occurred in 2.7% (2/75) of procedures. It occurred at day 3 and 21 after the index procedure, respectively. One patient was treated with pressure bandage and an external lumbar drain on day 4 after diagnosis of the leak, the other was treated with wound revision surgery on day 1 after the leak occurred. In total 1 patient developed a PMC without a CSF leak which was treated with wound revision surgery. SSI occurred in 10.7%, which included both cases of CSF leak.

Conclusions

CSF leakage after intradural spinal surgery in the pediatric population is relatively rare (2.7%). Nevertheless, the clinical consequences with respect to secondary complications such as infection and necessity for invasive treatment are serious.

Key Words

Cerebrospinal fluid leakage, pediatrics, infection, spinal surgery, spina bifida

Introduction

Cerebrospinal fluid leakage is a potentially serious complication after intradural spinal surgery. The complications associated with CSF leakage include wound infection, meningitis and CSF hypotension. CSF leakage may necessitate invasive treatment, such as surgical wound revision, and prolong hospitalization.[1, 2] In addition it is associated with increased health care costs.[1, 2]

Watertight closure is thought to be the most important step to prevent postoperative CSF leakage. Surgeons may make use of autologous or synthetic duraplasty material and may choose to use a sealant. Their efficacy in the prevention of CSF leakage, especially in the pediatric population, however, has not been studied.

There is variation in the definition of CSF leakage across different studies.[3] Some definitions of CSF leakage may include both incisional leakage and pseudomeningocele (PMC).[3] Yet, incisional CSF leakage is defined as leakage of CSF through the skin and PMC is a collection of CSF under the skin.[4] PMC is often self-limiting.[4] Therefore, in the current study we will define CSF leakage as incisional CSF leakage and consider PMC separately. Furthermore, reports on CSF leakage after intradural spinal surgery based on recent data are limited and mostly include a specific indication only.[5-11]

This study aims to establish the risk of CSF leakage and associated complications after intradural spinal surgery in three tertiary neurosurgical referral centers between 2015 and 2021 and to describe the treatment strategies applied. The results will be compared to the existing literature.

Evaluation of the up-to-date risk of CSF leakage and associated complications in a multicenter setting may serve as a benchmark and assist counseling of future patients and parents.

Methods

Study design

This is a multicenter retrospective cohort study of all consecutive pediatric patients in the Wilhelmina Children's Hospital and Princess Máxima Center for Pediatric Oncology in Utrecht, the Netherlands and the University Children's and University Hospital in Zurich, Switzerland. This study was approved by the institutional research boards and the local ethics committees and conducted according to the STrengthening the Reporting of OBservational studies in Epidemiology guidelines.[12]

Study population

All patients of 18 years or younger who underwent intradural spinal surgery between Janurary 1st 2015 and June 30th 2021 with a surgical report available were included. Patients who died within 6 weeks after surgery, were lost to follow-up or had reoperation within 6 weeks for other reasons than CSF-leakage treatment were excluded. Inclusion and exclusion criteria are presented in **Online Resource 1**.

Data collection

Procedures eligible for inclusion were screened based on a prospectively collected database or operation codes in the electronic patient records. Data was collected from the following sources: medical notes, medical imaging and surgical reports between 6 weeks prior to surgery and 6 weeks after surgery.

Surgical characteristics collected were: indication of surgery (tumor, vascular, developmental defect, trauma, infection), emergency surgery (yes/no), reoperation (defined as any consecutive surgery at the same anatomical location), the use of products with the intention to seal the dura (yes/no), duraplasty use (yes/no), attempt to watertight closure (yes/no), suture method used (continuous or standing) and suture material used.

The following patient data was collected: age at time of surgery, steroid use for multiple consecutive days directly before or after surgery, diagnosis of hydrocephalus based on medical imaging or medical notes, perioperative CSF diversion surgery (external ventricular drainage (EVD), external lumbar drainage (ELD), ventriculoperitoneal (VP)-shunt or endoscopic third ventriculostomy (ETV)), perioperative chemotherapy and perioperative radiotherapy. Patients who used steroids directly before or after surgery, or underwent perioperative chemotherapy, or were diagnosed with auto-immune disease were classified as immunocompromised.

Data items related to the clinical course included: total number of admission days, the number of days on the pediatric intensive care unit (PICU) and new neurological deficit after surgery (including worsening of existing deficit). If CSF leakage had occurred the following additional characteristics were retrieved: the estimated number of extra admission days due to CSF leakage (estimated based on treatment course for CSF leakage or readmission days), surgical wound revision because of CSF leakage, a CSF diverting procedure as a treatment for CSF leakage, the number of days of CSF diversion, lumbar puncture to treat CSF leakage, extra suture placement or pressure bandage because of CSF leakage, puncture of PMC.

The primary outcome measure of this study was CSF leakage within 6 weeks after surgery, defined as leakage of CSF through the skin (either confirmed with beta-2-

transferin test or reported in the clinical notes). Secondary outcome measures were: presence of PMC (defined as a subcutaneous collection of CSF), surgical site infection (SSI) (diagnosis or suspicion of SSI based on either antibiotic treatment according to the clinical notes, or a positive wound culture) and meningitis (suspicion or diagnosis of meningitis based on either antibiotic treatment according to the clinical notes, or positive CSF culture) within 6 weeks after surgery.

The PubMed and EMBASE database were searched until September 12, 2022 for studies reporting the CSF leakage incidence after spinal surgery in children. Studies including only one specific indication for surgery and case reports were excluded. The following search terms were used: ("cerebrospinal fluid leak*" OR "CSF leak*") AND ("pediatric" OR "peadiatric" OR "child*) AND ("spine" OR "spinal"). Reference lists of included articles were manually searched for additional relevant studies.

Statistical analyses

Baseline characteristics and incidence of secondary complications were presented as median with interquartile range (IQR) and compared between patients with CSF leakage and those without, using the Mann-Whitney U test for continuous variables and Fisher's Exact Test for categorical variables. Variables with ≥5% missing data were not included into statistical analyses. The primary outcome measure is presented the percentage of the total population. P-values <0.05 were considered significant. Because of the high number of potential risk factors for CSF leakage, Bonferroni correction was applied with a baseline significance level of 5%. All analyses were performed in IBM SPSS, version 26.0 (IBM Corp., Armonk, N.Y., USA).

Results

Demographics

We included 75 procedures, representing 66 individual patients (**figure 1**). The median age in this cohort was 5 (0-13) years, with a male preponderancy (n=43, 57.3%) (**table 1**). The most frequent indication was tumor resection (n=39), followed by developmental defects (n=34) and vascular surgery (n=2). Seventeen procedures were reoperations. Among the procedures for developmental defects were 11 myelomeningoceles, of which 1 had a preoperative leak (but no postoperative CSF leak).

In total 10 patients had undergone CSF diversion surgery of whom 5 preoperatively and 5 postoperatively. In total 1 patient who underwent tumor resection surgery had undergone preoperative ETV and 4 patients had a VP-shunt, of whom one patient was treated for a tumor and 3 for developmental defects. Another 5 patients received a VP-shunt postoperatively, all developmental defect cases with hydrocephalus.

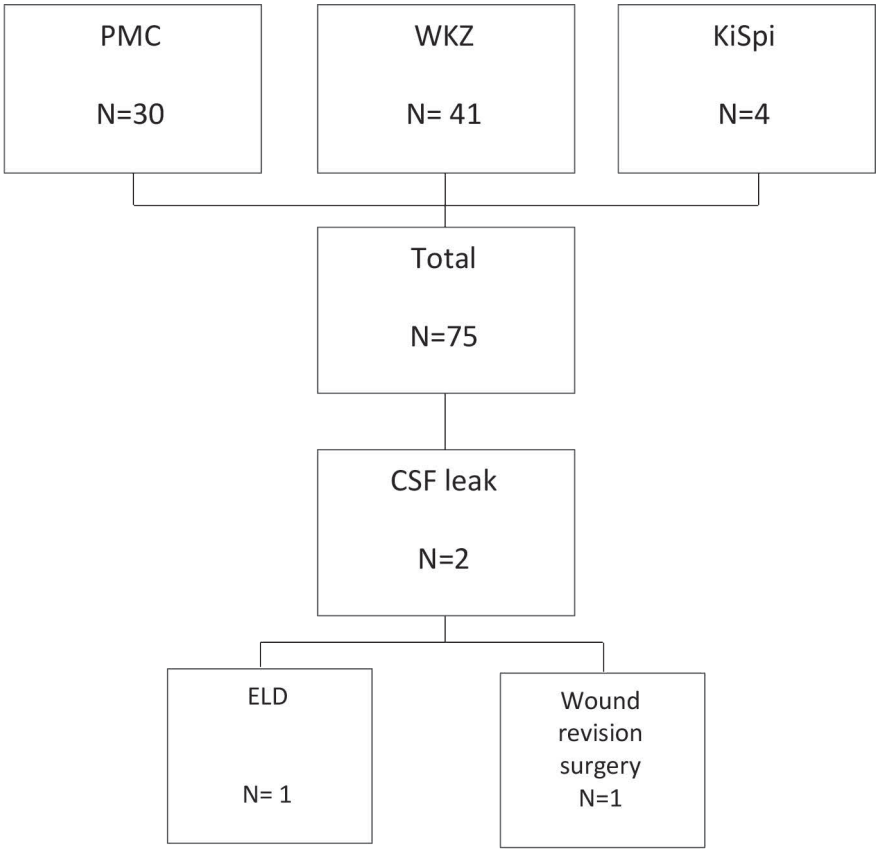

Figure 1. Flowchart of study population.

ELD: external lumbar drainage
KiSpi: University Children's Hospital Zurich
PMC: Princess Maxima Center for Pediatric Oncology
WKZ: Wilhelmina Children's Hospital

No statistically significant differences were observed between groups for the analyzed variables (**table 1**). There was a relatively high percentage of procedures with missing data for suture type, suture technique used and attempt of watertight closure (**table 1**).

Table 1. Study population characteristics.

Variable	Total (N=75)	No CSF leakage (N=73)	CSF leakage (N=2)	P-value
Patient characteristics				
Age, years	5 (0-13)	5 (0.5-13)	3 (0-3)	0.450
Sex (female)	32 (42.7)	32 (43.8)	0 (0.0)	0.504
Immunocompromised	18 (24.0)	18 (24.7)	0 (0.0)	1.00
Radiation therapy	9 (12.0)	9 (12.3)	0 (0.0)	1.00
Chemotherapy	10 (13.3)	10 (13.7)	0 (0.0)	1.00
Hydrocephalus	7 (9.3)	7 (9.6)	0 (0.0)	1.00
CSF diversion	10 (13.3)	10 (13.7)	0 (0.0)	1.00
Surgical characteristics				
Indication				0.025
Tumor	*39 (52.0)*	*39 (53.4)*	*0 (0.0)*	
Vascular	*2 (2.7)*	*1 (1.4)*	*1 (50.0)*	
Developmental defect	*34 (45.3)*	*33 (45.2)*	*1 (50.0)*	
Infection	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*	
Trauma	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*	
Reoperation	18 (24.0)	18 (24.7)	0 (0.0)	1.00
Emergency surgery	10 (13.3)	9 (12.3)	1 (50.0)	0.250
Sealant use	50 (66.7)	49 (67.1)	1 (50.0)	1.00
Duraplasty use	4 (5.4)	4 (5.5)	0 (0.0)	1.00
Autologous	1 (1.4)	1 (1.4)	0 (0.0)	
Non-autologous	3 (4.1)	3 (4.1)	0 (0.0)	
Watertight dura closure	71 (94.7)	70 (95.9)	1 (50.0)	NP
Suture material used				NP
PDS	38 (50.7)	38 (52.1)	0 (0.0)	
Vicryl	30 (40.0)	29 (39.7)	1 (50.0)	
Method of suturing				NP
Continuous	52 (69.3)	51 (69.9)	1 (50.0)	
Standing	1 (1.3)	1 (1.4)	0 (0.0)	
Clinical course characteristics				
Hospital stay, days	6 (4-10)	6 (4-9)	14.0 (11-14)	0.072
PICU stay, days	0 (0-1)	0 (0-1)	0.0 (0-0)	0.398
New/increased neurological deficit	12 (16.0)	12 (16.4)	0 (0.0)	1.00

All values are reported as numbers with percentages between brackets for categorical values and median with interquartile range (IQR) between brackets for continuous variables. Percentage of data missing: duraplasty use: 1.8%, watertight dura closure 5.3%, suture material 9.3%, method of suturing 29.3%. No data were missing for the remaining variables. Fisher's exact test was used for categorical variables and Mann Whitney-U test for continuous variables.
CSF: cerebrospinal fluid
N: number
NP: not performed
PDS: polydioxanone suture
PICU: intensive care unit

CSF leakage, associated complications and treatment

CSF leakage occurred in 2.7% (2/75) of procedures. The first patient was a male newborn who underwent an untethering procedure. CSF leakage occurred at day 21. The patient was readmitted and underwent wound revision surgery 1 day later. The total readmission period was 7 days. The second patient was a 6-year-old boy who underwent a vascular procedure, and the leak occurred at day 3 after the index procedure. A pressure bandage was applied and at day 4 after diagnosis of the leak an external lumbar drain (ELD) was placed.

The ELD could be removed after 6 days and wound leakage did not recur. Hospital stay was prolonged with an estimated 10 days. The index procedure was the first surgery in both cases and no dural grafts were used. The patients were not immunocompromised and had no hydrocephalus. In total 3 patients developed a pseudomeningocele or CSF leak (3.8%). There was one case of pseudomeningocele without a CSF fistula, which was treated with wound revision surgery. A total of 8 cases of SSI occurred (10.7%). Patients with a CSF leak had evidence for an increased risk for SSI, where 6 of 73 (8.2%) patients without, and both patients with CSF leak developed a SSI, respectively (p=0.01). One patient with an SSI (and no CSF leakage) was treated with surgical wound revision. All patients with SSI and CSF leakage received antibiotic treatment. There were no cases of meningitis is this cohort.

The CSF leakage risks in pediatric populations of intradural spinal surgery reported in the 4 studies identified in the literature range from 0-12.7% **(table 2)**.

Table 2. Literature overview of CSF leak after spinal surgery in the pediatric population.

Author	Year	N	CSF leak (%)	SSI (%)	Meningitis (%)
Kaufman et al.[8]	2010	27	0.0	0.0	0.0
Liu et al.[a, 9]	2014	638	7.1	3.1	0.0
Goodwin et al.[7]	2014	93	5.4	NR	1.1
Balasubramaniam et al.[5]	2014	102	12.7	NR	NR
Slot et al.[b]	2023	75	2.7	10.7	0.0

[a] Percentage is based on definition of CSF leakage including pseudomeningocele. The percentage of overt CSF leakage reported in this study is 2.8%.
[b] Represents the current study.
CSF: cerebrospinal fluid
N: number
NR: not reported

Discussion

The incidence of CSF leakage defined as leakage through the incision after spinal surgery in the pediatric population was 2.7%. To our knowledge this study is the first international, multicenter report of CSF leakage after intradural spinal surgery in children.

Definitions of CSF leakage in the current body of literature are not always described and may vary. Liu et al. reported a risk of 7.1%, which includes cases of PMC only as well. When looking at cases with incisional leakage in their large series of intradural spinal surgery specifically, they report 18/638 cases to have an overt CSF leakage (2.8%). This is comparable to the risk of incisional CSF leakage observed in our study (2.7%). The combined CSF leakage and PMC risk in our study is substantially lower: 3.8%. This discrepancy may be the result of the subjective definition of PMC, especially considering the retrospective nature of both studies. Another explanation may be the difference in distribution of procedures performed between these studies. Kaufman et al. found no CSF leakage in their study reporting on the use of non-penetrating anastomotic clips to close the dura after spinal procedures.[8] This is however a small series, which includes several procedures with dural incisions of 10 mm or less in length.[8] The use of a PEG-hydrogel was evaluated in another study, which reported a 5.4% CSF leak risk in procedures in which this type of sealant was used.[7] Two studies investigating the association between the use of fibrin glue and CSF leakage did not find a statistically significant effect.[5, 9] Currently, we believe that there is insufficient evidence to recommend the use of additional methods to augment dural closure.

To achieve optimal watertight closure, the first choice is to close the dura primarily using microsurgical techniques. If this is not possible, the use of autologous duraplasty material is second choice. The third choice would be to make use of an allogenic duraplasty. The low leakage rate observed in this spinal cohort is in line with the observation that for the largest group in this cohort, tumor resection cases, we generally find an intact dura to be opened surgically during the procedure, which we are able to close primarily, without the need for duraplasty. In the vast majority of cases this involves laminotomy with replacement of the lamina. For the other large group in our cohort, developmental disorders, we more often see a defect in the dura, for which a duraplasty has to be used. We advise to leave any subcutaneous lipoma in place, for proper closure of the skin tissue to reduce the relatively higher leakage risk in these cases.

The CSF leakage risk found in this spinal cohort is lower than that found in the cohort of intradural cranial surgery procedures (7.5%) in the same centers for this time period.[13] This also applies when compared to most previously reported CSF leakage risks in intradural spinal surgery (0-12.7%).[5,7,9] This contradicts the believe that the risk of CSF

leakage after spinal surgery is higher, due to increased intradural hydrostatic pressure in upright position at the surgical site on spinal level compared to cranially.[9]

The percentage of surgical site infections in our series is relatively high which may partially be explained by our lenient definition of SSI (which does not require a positive wound culture). Previous studies report SSI risks between 0.3-12.7%.[9, 14-17] Liu et al. find a significantly higher incidence of wound infection in patients with CSF leak compared to patients without in their study.[9] However, they have also included PMC only in the definition of CSF leak. All cases of wound infection in that group, though, occurred in patients with incisional leak, which confirms our rationale for considering PMC as a separate clinical entity. There were no cases of meningitis in our study, which is in line with low incidence of meningitis reported in other publications.[7,9,18]

Risk factors identified for CSF leakage in the study by Liu et al. after intradural spinal surgery in the pediatric population are: previous spinal surgery, the use of a dural graft, older age, use of non-locked continuous suturing and the procedure performed.[9] Cord untethering operations had the highest CSF leak risk in their study. This is consistent with the relatively high CSF leak risk reported in the majority of other series including tethered cord syndrome procedures specifically.[6,10,11,14,15,17] This can be explained by the inherent relation between the pathology in lipomyelomeningocele, often resulting in a dural defect at closure, and (myelo)meningocele and CSF leak.[11] Chern et al. find no association between CSF leak and surgery time, bedrest, use of a sealant or use of the microscope in their retrospective series among patients who underwent cord untethering procedures for tight filum terminale.[6] One out of 2 CSF leakage cases in our study was an untethering procedure as well.

CSF leakage was treated with invasive treatment (ELD and revision surgery, respectively) in both cases in our study. Balasubramaniam et al. also report invasive treatment with lumbar drainage, placed a few millimeters from the incision, preferably one or two levels above, in all procedures and reoperation in 23%.[5] Liu et al. report a reoperation risk of 44.4% for incisional CSF leakage, whereas the majority of pseudomeningoceles resolved without any intervention.[9] In our series the one case of PMC without CSF leak was treated invasively.

The most important limitation of this study is the small sample size and low number of CSF leaks (N=2), which limits the validity of statistical analyses performed. Furthermore, this retrospective study is vulnerable to reporting bias as it depends on existing clinical records. The variables with missing data for suture type, suture technique used and attempt of watertight closure reflects this bias. These variables could therefore not be incorporated into our statistical analyses. Furthermore, we did not collect data on bedrest prescription and level of the surgery. Yet, these factors may

actually be of influence on the risk of CSF leakage and should thus be further evaluated in future studies.

The comparison to previous literature was limited by the unclear and varying definitions in the existing body of literature. We suggest to use the definition of CSF leakage as "leakage through the skin" and consider PMC separately in future studies.

This study shows that CSF leakage after intradural spinal surgery in the pediatric population is rare (2.7%). Nevertheless, the clinical consequences with respect to complications such as infection, necessity for invasive treatment and prolonged hospitalization are serious. Future studies into the development of preventative strategies for spinal surgery in the pediatric population are warranted.

Declarations

Abbreviations

CSF: cerebrospinal fluid
ELD: external lumbar drainage
ETV: endoscopic third ventriculostomy
EVD: external ventricular drainage
IQR: interquartile range
PICU: pediatric intensive care unit
PMC: pseudomeningocele
SSI: surgical site infection
VP: ventriculoperitoneal

Ethics approval and consent to participate

This study was approved by the institutional research boards and the local ethics committees

Consent for publication

NA

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

Tristan van Doormaal is a consultant for Polyganics B.V.

Funding
Emma Slot receives a research grant through Polyganics B.V., a biotechnology company developing a dural sealant.

Authors' contributions
Eelco Hoving, Tristan van Doormaal and Emma Slot contributed to the study conception and design. Material preparation, data collection and analysis were performed by Eelco Hoving, Menno Germans and Emma Slot. The first draft of the manuscript was written by Emma Slot and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements
NA

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Supplemental table 1. Inclusion and exclusion criteria

Inclusion criteria
Intradural spinal surgery
≤18 years old at time of surgery
Surgical report available
Exclusion criteria
Death within 6 weeks after surgery
Lost to follow-up within 6 weeks after surgery
Reoperation for other reason than CSF leakage within 6 weeks after surgery

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Discussion

Safety and efficacy of Dura Sealant Patch in cranial surgery

Cerebrospinal fluid leakage is frequent and serious complication after neurosurgical intervention. In addition to health-related consequences of CSF leakage, patients with CSF leakage after intradural cranial surgery have substantially and significantly higher health care costs.[1] Improved preventative strategies reducing CSF leakage, even though they may add to the overall healthcare costs per patient, could be beneficial from an economic standpoint. However, whether the use of preventative measures that require financial input in all patients or a subgroup of patients at risk results in cost savings depends on their price and efficacy. The results of this economic evaluation of CSF leakage costs shows that there is a need for preventative strategies to reduce CSF leakage not only from a patientcare perspective, but from a health-economic perspective as well. The model developed in this study may assist hospitals in the Dutch setting in decision making with regards to which preventative strategy to invest in, taking into consideration efficacy and price.

The safety and efficacy of the new dural sealant, Dura Sealant Patch (DSP) (Liqoseal®, Polyganics B.V. Groningen, The Netherlands), to reduce CSF leakage after cranial surgery in adults is currently being investigated in a randomized, two-armed, multicenter trial (ENCASE II).[2] This study was designed as a non-inferiority trial using two commonly used PEG-hydrogel sealants as control. Preliminary safety analysis in the first 30 subjects does not show any device related (serious) AEs. The final follow-up is expected to be completed in November 2023. Recommendations based on for which patient group this novel sealant would be cost effective can only be made upon completion of the trial.

Safety and efficacy of Dura Sealant Patch in trans-sphenoidal surgery

The CSF leakage risk after transsphenoidal surgery (TSS) is considerable with a 3.4% overall risk and higher risk for intradural and invasive lesions such as craniopharyngiomas or tuberculum sellae meningiomas.[3] TSS is regarded as a form of cranial surgery, and thus DSP application is not off-label. However, the surrounding tissue and dimensions in this approach are different compared to a craniotomy. Therefore we have evaluated the application of DSP in TSS in preclinical (*ex vivo*) setting and 3 endoscopic transsphenoidal cases.[4] In these cases DSP was applied upon discretion of the operating surgeon as a salvage treatment. The overall mean burst pressure of DSP in the *ex vivo* transsphenoidal model and mean burst pressures in individual groups based on compression weight and time were all well above physiological intracranial pressure.[4] Mean burst pressure in this model was shown to be similar to those found

in our cranial and spinal model.[5] DSP was successfully applied transsphenoidally in 3 patients. We found no indications of safety issues for the transsphenoidal application of DSP based on these 3 patients.[4 3] The results of this thesis combined with the outcomes of the ENCASE trial[6,7] and previous preclinical studies with regard to CSF leakage[5,8-13], indicate that the use of DSP in the sphenoid sinus to seal a dural defect in TSS is likely safe and potentially effective. This novel preventative measure is especially useful in high-risk cases. Yet, a prospective randomized controlled trial is required to establish safety and efficacy of DSP implantation as compared to the use of NFS and/or other sealants, also taking into consideration the use of preventative external lumbar drainage, in TSS.

Safety of Dura Sealant Patch in spinal surgery

The use of DSP has not been approved for spinal surgery. To this end an animal model was developed for preclinical evaluation of DSP application in spinal surgery. An *in vivo* porcine model for interlaminar decompression was designed. Throughout the development of this model, it was noted that fixation of the spine with lubra plates (Veterinary Orthopedic Implants Inc, St. Augustine, USA) may prevent neurological deficits.[14] The histological and MRI evaluation of the pigs implanted with DSP was compared to data from a cranial porcine model of similar survival time. The spinal dural histological reaction to DSP during the first 7 days was similar compared to the cranial dural reaction. DSP does not significantly swell in both application areas over time. Furthermore, no safety issues were reported in the first in human cranial study (ENCASE).[6,7] Combined with previous data, the findings in this thesis suggests that DSP can be safely applied on spinal dura.

Cerebrospinal fluid leakage in children

Despite, CSF leakage being a well-known complication after neurosurgical intervention the exact magnitude and risk factors in the pediatric population were still largely unknown. We performed a meta-analysis to evaluate the risk of CSF leakage after cranial surgery in children and found a 4.4% overall risk.[15] The main limitation of this study was the lack of a uniform definition of CSF leakage across the available literature. Therefore, we subsequently investigated the risk of CSF leakage defined as incisional leakage within 6 weeks after surgery in a historical cohort study. We found that the risk of CSF leakage after trepanation in children is significant (7.5%), comparable to the risk reported in adults, and has a serious clinical consequences.[16,17] Hydrocephalus and craniectomy are potential risk factors, after correcting for infratentorial surgery, emergency surgery, immunocompromised status and chemotherapy.[16] The risk of CSF

leakage after intradural spinal surgery in children, however, is considerably lower (2.7%).[18] Yet, this study was limited by the small absolute number of cases included[18]. Despite the comparable risk of CSF leakage after cranial surgery between children and adults, no prospective studies investigating commonly used dural sealants in pediatric patients have been published.[16,17]

It is a common trend throughout medical drug and device research that trials in pediatric patients are lagging behind. Only 12% of trials registered on clinicaltrials.gov is for the pediatric population, despite the contribution of this population to the total disease burden being 60%.[19] As a result the majority of medicines and devices used by children world-wide are off-label, without information on dosing, safety and efficacy available for this specific patient population.[19–21]

For various dural sealants clinical trials have been performed in adults leading to market approval[7,22-24], yet there is a paucity of prospective studies in children. Only one pediatric trial investigating the safety and efficacy of Evicel (Johnson and Johnson, New Brunswick, USA) in obtaining watertight dural closure as compared to additional suturing in patients undergoing cranial surgery has been registered. The commonly used polyethylene glycol (PEG) hydrogel sealants DuraSeal (Integra LifeSciences, Princeton, USA) and Adherus (Stryker, Kalamazoo, USA) are only indicated for use in children from the age of 13 years old. TachoSil (Corza Health, San Diego, USA) has been CE approved for the purpose of dural sealants in adults, whereas in children its use has been investigated in children for the treatment of local bleeding in liver resection surgery only.[25]

The paucity of pediatric trials may result from insufficient funding. Lack of funding from the industry for pediatric trials may be explained by multiple factors; decreased commercial interest, increased costs and greater risk of liability and more restrictive regulatory oversight.[19] Despite the common perception of reduced market potential, the pediatric healthcare market is forecasted to grow to 15,984 million US dollars by 2025.[26] Currently, however, pediatric trials are more reliant on non-profit funds or governmental funds.[19] Furthermore, physicians are also more hesitant to involve children in clinical trials out of fear of uncertain treatment effects. In addition the burden of participating in a clinical trial may be different for children compared to adults. Pragmatic trials in which routine clinical tests and exams are used are thus to be preferred.[19,20] Another hurdle in pediatric trials are the smaller sample sizes and population heterogeneity which complicates recruitment.[27] Study designs may need to incorporate stratification by age, because of physiological changes that coincide with development throughout childhood.[26] This affects the sample size required to adequately power such study.[26]

A recent study argues that for device research in pediatrics, availability and technological sophistication falls behind by a decade compared to the adult population.[27] The USA has legislation to stimulate investigations for both pediatric drug and medical device research. Additional efforts were made by dedicating funding and the formation of expertise networks specifically for pediatric research.[19] In Europe such regulations only exist for pediatric medicines.[26] There are various national initiatives to promote medical device development for children in the form of collaborations networks, though.[26] Yet, despite initiatives, such as governmental funding through the pediatric device consortia program in the USA, to stimulate innovation in pediatric medical device research, efforts from all stakeholders including industry and academia are necessary to counteract this inequality.[27] The European Pediatric Translational Research Infrastructure connecting all stakeholders and offering support to the research community is an important step towards this goal.[26]

Although there is a lack of prospective studies investigating safety and efficacy of dural sealants in the pediatric population, retrospective analyses are available. Zhou et al. (2014) have evaluated the use of PEG sealants in 163 patients aged 0-18 years who underwent cranial procedures.[28] They conclude the use of PEG sealants in the pediatric population is safe, based on the low incidences observed for CSF leakage (1.2%), meningitis (0.6%) and superficial skin infection (2.4%). In a series of spinal neurosurgical cases Goodwin et al. (2014) evaluated the use of a PEG hydrogel in addition to standard methods of dural suturing in 93 spinal procedures in children.[29] The CSF leakage risk observed was 5.4%, with a meningitis risk of 1.1%. There were no deaths or associated neurological deficits. The authors conclude that based on these results the use of these sealants in pediatric cases appears to be safe. Parker et al. investigated complications in a series of pediatric patients who underwent decompression for Chiari Malformation.[30] In their series different sealants were used in the majority of cases to augment watertight closure of the duraplasty used. A total of 114 patients received various combinations of duraplasty (EnDura (Integra LifeSciences, Princeton, USA), Durepair (Medtronic, Dublin, Ireland) or cadaveric pericardium) and a sealant (Tisseel (Baxter, Deerfield, USA), DuraSeal (Integra LifeSciences, Princeton, USA)) or duraplasty without a sealant. The overall incidence of CSF leakage requiring surgical intervention in this series was 5.2%. There was a significant difference in complication rates depending on the type of graft used and whether a sealant was added. Contrary to Zhou et al. (2014)[28] and Goodwin et al. (2014)[29] this study concludes that the addition of a sealant may not be beneficial, as the lowest complication rate occurred in patients that did not receive a sealant.[30] Results from retrospective pediatric studies investigating sealant use are thus inconsistent.

The level of evidence for the majority of now commonly used off-label sealants in children is low. The most important limitation of the currently available evidence with regard to the use of sealants is the retrospective nature of the studies. Sealants may have been applied selectively to cases with high risk only. Furthermore, they are subject to reporting bias and may not have captured all potential adverse events related to the use of sealants. Taking into considerations that logistic and financial constraints of performing a randomized controlled trial, at least single-arm prospective trials are necessary to establish a safety profile for the use of commonly used sealants in the pediatric population.

Evaluation of the use of DSP in pediatric patients has not yet started. Given the similar incidence rate of the complication in children compared to adults, further development of this device for use in children would meet a clinical need. Especially, since no other devices have been approved in this population to reduce CSF leakage. To evaluate safety of the use of DSP in children (1-17 years old) a single-arm open label trial should be performed similar to the adult ENCASE trial. In line with recommendations from Joseph et al. (2013) this trial should have a pragmatic design, and evaluations should be based on standard of care where possible.[19] Follow-up visit windows should be based on clinical follow-up, thus include a visit at day 7 or discharge (whichever comes first) when the patient is hospitalized and regular postoperative outpatient clinical follow-up around day 40. The final clinical follow-up should coincide with the MRI follow-up. The diagnosis of CSF leakage should be based on physicians' suspicion without confirmation through beta-2-transferrin testing if not clinically necessary to avoid additional blood test for research purposes. Although undergoing an MRI is burdensome for pediatric patients this procedure is necessary for proper evaluation of safety as it will provide insight into the potential swelling and complications such as pseudomeningocele. Inclusion of patients that will undergo MRI follow-up within the first 7 days and at 90 days postoperatively as part of their standard of care is thus preferential. The 12 months follow-up necessary to follow patients for the maximum degradation period of the product could be performed through telecommunication.

Based on our recent historic cohort study we expect 10% of pediatric patients to meet one or more composite primary endpoints (incidence of wound infection or meningitis, incidence of CSF leakage, incidence of pseudomeningocele with the need for intervention).[6] Allowing for a confidence interval with a width of 20% this would require a sample size of 47 patients (including a 10% drop-out rate). A trial design without additional visits would benefit recruitment as disruption of daily life is one of the most mentioned discouraging factors for participation in research by both children and parents.[20] Pediatric patients should also be involved in the design of the

trial, as they can provide valuable insights into research participation.[26] Active patient participation in research has been a trend throughout Europe for the last 10 years.[26]

The biological safety evaluation on which the preclinical studies have been based reveals a maximum size of 10x16 cm for adult females (reference weight 58 kg). Extrapolating this to the body mass recommended by the ISO 10993-17 for children aged 1-16 years assuming a weight of 10 kg would allow for implantation of a patch with a maximum surface of 27.6 square cm.[31] A 5x5 cm patch, which is below the maximum recommended surface area, is already commercially available. The actual size of the patch to be implanted will likely be smaller in the vast majority of cases as the device will be cut to size prior to application.

Although, the results of randomized-controlled trial would be able to provide insights into both safety and efficacy of DSP as compared to current best practice in children this may not be feasible for logistic and financial reasons. To avoid off-label use of the device in children without knowledge of potential safety issues, however, a single-arm safety trial would be a valuable first step.

Limitations

This thesis is subject to the following limitations. Firstly, the health economic evaluation of CSF leakage costs is based on one center in the Netherlands. Therefore, generalizability of these results is limited. The results of the ENCASE II trial comparing the novel Dura Sealant Patch, DSP, to two commonly used comparators are not complete yet. Thus it remains unknown whether DSP performs non inferior to control. Moreover, DSP is compared to PEG-hydrogel sealants in this study only, as these are both Food and Drug Administration (FDA) and Conformitè Européenne (CE) approved. In Europe, Tachosil is often used as a sealant in neurosurgical procedures. The ENCASE II trial, however, will not provide insight into the efficacy of DSP compared to Tachosil, despite its relevance in European neurosurgical practice. The most important limitation of both the animal studies on spinal application of DSP and the case series on transsphenoidal application of DSP is the small sample size of these studies.

Similarly, our evaluation of the incidence and risk factors of CSF leakage in the pediatric population is limited by the low number of cases. Although our evaluation of cranial cases to our knowledge is the largest cohort to date, the absolute number of cases with CSF leakage remains low. For our spinal analysis the sample size of the cohort as well as the absolute number of cases with CSF leakage is limited, despite the multicenter approach of this study. Larger collaborations are necessary for evaluation of this specific surgical population.

Potential future directions

The findings in this thesis confirm the clinical need for effective preventative strategies to reduce CSF leakage from a health care and economic perspective, in both adults and children. Preventative strategies may be beneficial from a health-economic point of view as well. Furthermore, it is shown that the use of DSP in TSS is safe and potentially effective. Based on the studies in this thesis the following recommendation for further research can be made. Firstly, given the current lack of safe and effective dural sealant for spinal surgery and burden of disease caused by CSF leakage, we propose that an in-human investigating safety and efficacy of DSP in spinal surgery in adults should be performed.

Secondly, further development of DSP for cranial pediatric use is warranted given the incidence of CSF leakage in the pediatric population and lack of alternatives available.

Thirdly, a post-market surveillance program is recommended for cranial use of DSP, both for trepanations and transsphenoidal surgery. Data should be gathered to compare the efficacy of DSP to Tachosil, a commonly used dural sealant in Europe currently which has not been investigated in the ENCASE II trial. In addition, safety and efficacy data should be collected for transsphenoidal cases to compare the efficacy of DSP to the bench mark of best standard of care considering its use in TSS is not off-label despite the lack of a prospective trial investigating its safety and efficacy.

In addition to future research efforts, the success of preventative strategies against CSF leakage is depended on the neurosurgeons making use of them. Firstly, neurosurgeons need to be informed about the problem of CSF leakage. Secondly, neurosurgeons need to be aware of the improvements of the novel strategies offered.

Therefore, presenting our research on why CSF leakage should be prevented both from a patientcare and health-economic perspective, who are most at risk, and what the potential role of various preventative strategies is, is only the starting point. Implementation of novel preventative strategies requires reflection within neurosurgical departments on their specific CSF leakage cases, prevention strategies currently applied and a joint effort to commit to attempting to improving current rates. As multiple individual preventative strategies may exist in neurosurgical departments, the research presented in this thesis may help departments in adopting a more uniform strategy. Moreover, learning by example of pioneers in the field plays a pivotal role in implementing novel techniques for the next generation.

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11

Summaries

Summary

This Ph.D. thesis describes further applications of a novel dural sealant patch to prevent cerebrospinal fluid leakage. The cerebrospinal fluid physiology and consequences of CSF leakage as well as current knowledge with respect to preventative strategies are described in the **introduction (chapter 1)**. Cerebrospinal fluid leakage is a well-known complication after neurosurgical procedures. The dura mater may have been compromised unintentionally or have been opened intentionally during such procedure. Despite meticulous closure with suturing after intentional incision of the dura, leakage of cerebrospinal fluid through the skin may occur. To mitigate the risk of CSF leakage a novel medical device to prevent CSF leakage, Dura Sealant Patch (DSP) (Liqoseal®, Polyganics B.V. Groningen, The Netherlands), was developed to augment watertight dural closure after suturing.

Part I of this thesis discusses the prevention of CSF leakage in cranial surgery in adults. The **second chapter** of this thesis describes the health-economic consequences of CSF leakage after cranial surgery from a hospital perspective in the Netherlands. Mean cost difference between patients with and without CSF leakage was €9,665 (95%-Confidence Interval (CI), €5,125 to €14,205). Main cost driver was hospital stay with a difference of 8.5 additional days in hospital for patients with CSF leakage. This study furthermore modelled the potential cost savings if CSF leakage were to be reduced. A maximum cost reduction of -€653,025 (95% CI -€ 1,204,243 to -€169,120) per 1,000 patients could be achieved if CSF leakage would be reduced with 75% in all patients, with 72 cases of CSF leakage avoided.

The protocol of a randomized-controlled trial evaluating the safety and efficacy of DSP as compared to standard of care, which is currently carried out, is presented in **chapter 3**. This study aims to recruit 228 patients undergoing elective infratentorial surgery and is designed as a non-inferiority trial.

The **second part** of this thesis is directed at prevention of CSF leakage after transsphenoidal surgery. The meta-analysis described in **chapter 4** provides a bench mark of the incidence of CSF leakage after transsphenoidal surgery (3.4%), a form of cranial surgery. CSF leakage is more frequent when intra-operative CSF leakage occurs and when cavernous sinus invasion is present. In **chapter 5** the results of *ex vivo* experiments of application of DSP in a transsphenoidal procedure and application of DSP as a salvage treatment in 3 patients are presented. The burst pressure of DSP in this transsphenoidal model is well above physiological intracranial pressure. DSP was applied during transsphenoidal procedures with intraoperative CSF leakage to prevent postoperative CSF leakage. None of the patients had a postoperative CSF leakage. No nose passage problems were observed.

Part III of this thesis focuses on safety of application of DSP on spinal dura. The **6th chapter** describes how we adapted a spinal in vivo porcine model by fixating the spine with Lubra plates to avoid neurological deficit. This animal model was used to evaluate safety of spinal implantation of DSP as described in **chapter 7**. The comparison of spinal MRI and histological data showed similar reactions to DSP as a previous cranial porcine model. These results lay the foundations for a first in human study for spinal application.

Part IV of this thesis consists of studies evaluating incidence and risk factors of CSF leakage in the pediatric patients. These chapters provide the groundwork for future studies investigating preventative strategies in this population. **Chapter 8** presents the results of a systematic review and meta-analysis and concluded that definitions of CSF leakage are underreported and are very variable. This results in a wide interval of presumed risk of CSF leakage in children (0-38%) with an average risk of 4.4%. **Chapter 9** and **chapter 10** describe two subsequent multi-center, international, historical cohort studies including cranial cases and spinal cases, respectively. We found that the incidence of CSF leakage after cranial surgery in children is comparable to that in adults (7.5%). Craniectomy and hydrocephalus were identified as independent risk-factors. CSF leakage after intradural spinal surgery, on the other hand, was relatively rare (2.7%). Nevertheless, the clinical consequences with respect to secondary complications such as infection, necessity for invasive treatment and prolonged hospitalization are serious. Despite the clinical necessity for preventative solutions for the pediatric population, clinical evidence for this specific population is lacking for the most commonly used dural sealants.

The findings presented in this thesis indicate the need for effective preventative strategies for CSF leakage. Such preventative strategies may also be beneficial from an economic perspective. DSP proofs safe and potentially efficacious for cranial use, including transsphenoidal procedures, in adults. Furthermore, this thesis has laid the ground work for future clinical studies for spinal use and use in pediatric population.

Nederlandse samenvatting

In dit proefschrift worden verdere toepassingen van een nieuw medisch hulpmiddel ter preventie van hersenvochtlekkage beschreven. In **hoofdstuk 1** wordt een inleiding gegeven van de fysiologie van hersenvocht, consequenties van hersenvochtlekkage en de huidige kennis op het gebied van preventieve strategieën. Hersenvochtlekkage is een bekende complicatie na neurochirurgische operaties. Het hersenvlies kan per ongeluk of als onderdeel van de operatie worden geopend. Ondanks zorgvuldig hechten van het hersenvlies kan het doorbreken van deze anders waterdichte barrière leiden tot lekkage van hersenvocht door de wond. Om het risico hierop te beperken is een nieuw medisch hulpmiddel, *Dura Sealant Patch* (DSP) (Liqoseal®, Polyganics B.V. Groningen, Nederland), ontwikkeld.

Het **eerste deel** van dit proefschrift behandelt de preventie van hersenvochtlekkage in craniale chirurgie bij volwassenen. In **hoofdstuk 2** worden de gezondheidseconomische gevolgen van hersenvochtlekkage na craniale chirurgie beschreven vanuit het ziekenhuisperspectief in Nederland. Het gemiddelde kostenverschil tussen patiënten met hersenvochtlekkage en patiënten zonder hersenvocht lekkage was €9,665 (95%-Betrouwbaarheidsinterval (BI), €5,125 tot €14,205). De belangrijkste kostendrijver was ziekenhuisopname met een gemiddeld verschil van 8.5 dagen. In dit onderzoek werd verder gemodelleerd wat de mogelijke kostenbesparing zou kunnen zijn als hersenvochtlekkage zou worden verminderd. Een maximale kostenreductie van -€653,025 (95% BI -€ 1,204,243 tot -€169,120) per 1,000 patiënten zou kunnen worden gerealiseerd bij 75% vermindering in alle patiënten, waarbij 72 gevallen van hersenvochtlekkage zouden worden voorkomen.

Het protocol van een gerandomiseerde gecontroleerde studie naar de effectiviteit van DSP ten opzichte van de goudenstandaard ter voorkoming van hersenvochtlekkage na craniale chirurgie bij volwassenen, die heden wordt uitgevoerd, wordt in **hoofdstuk 3** gepresenteerd. In deze studie worden 228 patiënten die electieve infratentoriële chirurgie ondergaan geïncludeerd. De studie is ontworpen als *non-inferiority* studie.

Het **tweede deel** van dit proefschrift behandelt preventie van hersenvochtlekkage na transsfenoïdale chirurgie. In **hoofdstuk 4** wordt ingegaan op toepassing van DSP bij transsfenoïdale chirurgie, een specifieke vorm van craniale chirurgie. In een meta-analyse wordt de maatstaf voor de incidentie van hersenvochtlekkage bij transsfenoïdale chirurgie (3.4%) weergegeven. Uit dit onderzoek blijkt het risico op hersenvochtlekkage verhoogd in geval er sprake is van hersenvochtlekkage ten tijde van de ingreep en ingeval van invasie van de sinus cavernosus. In **hoofdstuk 5** worden de resultaten van ex vivo experimenten van de applicatie van DSP bij een transsfenoïdale procedure en 3 casus beschreven. De barst druk van DSP in dit transsfenoïdale model ligt ruim boven de fysiologische intracraniale druk. DSP werd gebruikt tijdens

transsfenoïdale chirurgie bij 3 patiënten met intra-operatieve hersenvochtlekkage om postoperatieve hersenvochtlekkage te voorkomen. Geen van de patiënten had postoperatieve hersenvochtlekkage. Er werden geen neuspassage klachten gemeld.

Het **derde deel** van het proefschrift behandelt de veiligheid van applicatie van DSP op spinale dura. In **hoofdstuk 6** wordt beschreven hoe we een in vivo varkens model voor spinale chirurgie hebben ontwikkeld. Het model werd aangepast door het toevoegen van Lubraplaten om de wervelkolom te stabiliseren en zo neurologische uitval te voorkomen. Dit dierproefmodel werd gebruikt voor onderzoek naar de veiligheid van DSP bij spinale chirurgie, wat in **hoofdstuk 7** wordt beschreven. De MRI en histologie resultaten zijn vergelijkbaar met die uit een eerder craniaal varkensmodel. Er waren geen aanwijzingen voor veiligheidsbezwaren. Deze resultaten vormen het grondwerk voor een eerste studie naar spinaal gebruik van DSP bij mensen.

Deel IV van dit proefschrift richt zich op de preventie van hersenvochtlekkage bij kinderen. De incidentie en risicofactoren van hersenvochtlekkage bij kinderen zijn nog niet eerder uitgebreid beschreven. De hoofdstukken in dit deel kunnen de basis vormen voor verder onderzoek naar preventieve strategieën in deze doelgroep. **Hoofdstuk 8** beschrijft de resultaten van een systematisch literatuuronderzoek en meta-analyse waarin werd geconcludeerd dat de definities van hersenvochtlekkage onder-gerapporteerd en erg verschillend zijn. De resultaten lieten een breed interval van de veronderstelde incidentie zien (0-38%), met een gemiddeld risico van 4.4%. In een daaropvolgende cohort studie in internationaal verband, beschreven in **hoofdstuk 9**, werd aangetoond dat het risico op hersenvochtlekkage bij kinderen vergelijkbaar is met dat bij volwassenen (7.5%). Craniectomie en hydrocefalie werden als onafhankelijke risicofactoren geïdentificeerd. **Hoofdstuk 10** beschrijft een cohort studie in dezelfde centra met spinale casus. Hersenvochtlekkage na intradurale spinale chirurgie bleek relatief zeldzaam (2.7%). Desalniettemin, zijn de klinische gevolgen met betrekking tot complicaties zoals infectie en de noodzaak tot invasieve behandeling en verlenging van ziekenhuisopname ernstig. Ondanks de klinische behoefte aan preventieve oplossingen voor de pediatrische populatie, is er een gebrek aan klinisch bewijs over het gebruik van gangbare medische hulpmiddelen voor deze patiëntengroep.

De bevindingen die worden gepresenteerd in dit proefschrift geven weer dat er behoefte is aan effectieve preventieve strategieën tegen hersenvochtlekkage. Deze strategieën kunnen ook vanuit gezondheidseconomisch perspectief gunstig zijn. DSP is veilig voor craniaal gebruik bij volwassenen. De effectiviteit ten opzichte van huidige beschikbare *sealants* wordt onderzocht in een multicenter gerandomiseerde klinische studie. Daarnaast werd in dit proefschrift de basis gelegd voor verdere klinische studies naar gebruik van DSP bij spinale chirurgie en bij kinderen.

A

Appendices

I List of publications

II Contributing authors

III Acknowledgements

IV Curriculum Vitae

I List of publications

All publications of Emma M.H. Slot are reported in reverse chronological order listed as publications included in this thesis and publications not included in this thesis.

Publications included in this thesis

- **Slot EMH**, Colmer N, Serra C, Holzmann D, Regli L, van Doormaal TPC. Ex vivo and in vivo evaluation of transsphenoidal Liqoseal application to prevent cerebrospinal fluid leakage. Acta Neurochir. 2023. doi: 10.1007/s00701-022-05477-3. Epub ahead of print.
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Publications not included in this thesis

- Kinaci A, **Slot EMH**, Kollen M, Germans MR, Sepideh A-H, Carlson AP, Majeed K, Depauw PRAM, Robe PA, Regli L, Charbel FT, van Doormaal TPC. Risk Factors and Management of Incisional Cerebrospinal Fluid Leakage After Craniotomy: A Retrospective International Multicenter Study. Neurosurgery. 2023 Jun 1;92(6):1177-1182. doi: 10.1227/neu.0000000000002345. Epub 2023 Jan 23. PMID: 36688661; PMCID: PMC10158880.
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III Acknowledgements

Mijn dank gaat uit naar alle patiënten, die deel uit maken van dit onderzoek, als proefpersoon, door hun data beschikbaar te stellen en als bron van motivatie om steeds maar meer te weten te willen komen binnen de geneeskunde.

Allereerst wil ik mijn copromotor, **dr. Tristan van Doormaal** bedanken. Lieve Tristan, bedankt voor de kans die jij mij gaf om aan dit onderzoek te beginnen. Ik ben dankbaar voor het vertrouwen dat jij mij vanaf de start hebt gegeven, waarbij ik de vrijheid kreeg om overal mijn eigen weg in te zoeken, en tegelijkertijd wist dat je er was, mocht het nodig zijn. Ik heb ontzettend veel van je geleerd op gebied van onderzoek doen, dokter zijn, maar misschien nog wel het meest als mens.

Natuurlijk gaat ook mijn dank uit naar mijn promotor, **prof. dr. Eelco Hoving**. Eelco, hartelijk dank voor het voorbeeld dat je voor mij bent als arts en onderzoeker. Jij wist altijd op een hoffelijke manier een wijze raad te geven wanneer nodig, of een blijk van waardering om mij verder te motiveren.

Geachte leden van de beoordelingscommissie, **prof. dr. P.A.J.T. Robe, prof. dr. D. Salvatori, prof. dr. J.J. Verlaan, prof. dr. J.A. Grotenhuis, dr. M.L.C. van Veelen**, hartelijk dank voor het lezen en beoordelen van mijn proefschrift.

Beste coauteurs, van harte bedankt voor jullie bijdragen aan dit proefschrift en de prettige samenwerking. In het bijzonder dank aan **Ahmet, Menno, Kirsten, Sander en Saskia** voor jullie hulp bij het opzetten en uitvoeren van verschillende studies in dit proefschrift en het delen van jullie kennis.

Rengin, dank voor jou inzet, initiatief en leergierigheid tijdens jouw wetenschapsstage, waarvan het eindverslag heeft als basis heeft gediend voor een van de hoofdstukken in dit proefschrift.

I would like to thank everyone involved in the ENCASE II trial for their contributions. It was a joy to meet so many of you during the site initiation visits, albeit virtually. Special thanks to the Polyganics team, **Bart-Jan, Reinhardt, Raoul, Romke, Hilde** en **Carola**, for the excellent cooperation and everything you taught me.

Berber, jij was mijn steun en toeverlaat voor de ENCASE II studie in Utrecht. Dank voor alle praktische kennis die je mij bijbracht over onderzoek doen, en de administratie die daarbij komt kijken. Het was fijn om even te kunnen ventileren over de zoveelste net gemiste patiënt voor de studie of het volgende formulier wat ingevuld moest worden.

Daarnaast wil ik alle collega's van de afdeling Neurochirurgie bedanken voor de fijne samenwerking in de kliniek en tijdens mijn onderzoek. In het bijzonder gaat mijn dank

uit naar **prof. dr. Pierre Robe**, van wie ik veel geleerd heb op gebied van patiëntenzorg. Pierre, dank dat je altijd openstaat voor een gesprek over hoe het gaat, natuurlijk met een kopje Nespresso erbij.

Medical Business bestuur, ik wil jullie bedanken voor de levendige discussies, memorabele quotes, gezellige avonden, welkome afleiding en vooral het bewijs dat jullie leveren dat geneeskunde zoveel meer is dan arts(-onderzoeker) zijn alleen.

Lieve vriendinnen, ik ben dankbaar dat ik omringd ben door zoveel sterke vrouwen in mijn leven. Jullie zijn voor mij allemaal op jullie eigen manier een voorbeeld, bron van inspiratie en steun.

Irene, Milan en **Cynthia**, sinds de tijd dat een outfit kiezen voor het schoolfeest de grootste zorg was, en met wie sindsdien alles bespreekbaar is, en altijd vertrouwd voelt.

Tanya, Plym, Femke en **Lieke** met wie ik samen volwassen werd in de *dorms* van Amsterdam University College.

Ilja, **Iris** en **Lisa** met jullie werd ik samen dokter, en was zelfs wonen in Apeldoorn tijdens onze coschappen leuk.

Hannah en **Dagmar**, bazen, bedankt dat jullie altijd dichtbij mij staan, ook tijdens de moeilijke momenten, bedankt voor jullie eerlijke advies en vermogen om alles met een goed glas wijn te relativeren. Jullie vriendschap is goud waard.

En lieve vrienden, natuurlijk ook dank aan jullie.

Lieve, **Dorine**, paranimf, wij hebben onze hele studietijd samen doorlopen. Al die jaren heb ik het genoegen gehad om met jou prikkelende discussies te mogen voeren, te kunnen lachen en om bij jou mijn hart te luchten tijdens colleges, borrels en de ontelbare treinreizen tussen Amsterdam en Utrecht. Mijn studie is dan nu echt klaar, maar ik weet zeker dat ik de rest van mijn leven van mijn vriendschap met jou zal blijven leren en vooral genieten. Dank dat jij mijn paranimf wil zijn!

Lieve (schoon)familie, in het bijzonder mijn schoonouders, **Rik** en **Marjan**, dank voor jullie oprechte interesse in mijn onderzoek (en alles dat mij verder bezighoudt), jullie appjes voor een presentatie, felicitaties bij een publicatie en bovenal vertrouwen in mij.

Lieve, **Lucas**, ik vind het ontzettend bijzonder dat je mijn paranimf wil zijn, nog veel bijzonderder vond ik het om in september 2022 hetzelfde voor jou te mogen doen. Je bent dan wel jonger, maar in veel opzichten toch echt mijn grote broer en voorbeeld. Dank dat je altijd voor mij klaar staat en weet dat ik op ieder moment ook klaar sta voor jou.

Lieve **Jesse**, liefde van mijn leven, er zijn geen woorden om uit te drukken wat jij voor mij betekent en hoe belangrijk jouw steun tijdens mijn PhD-traject is geweest. Jij geeft mij het gevoel dat ik alles kan en tegelijkertijd dat falen niet erg hoeft te zijn. Jij maakt mij de gelukkigste vrouw van de wereld en ik ben trots dat jij mijn man bent.

Lieve **mama** en **papa** dank voor jullie onvoorwaardelijke geloof in alles wat ik doe, jullie grenzeloze steun, voor het warme thuis dat ik nog steeds bij jullie vind en vooral voor alles dat jullie mij geleerd hebben.

Mama, van jou leerde ik overal voor open te staan, klaar te staan voor een ander, en door te zetten. Weet dat ik net zo trots ben op jou als jij op mij.

Papa, van jou leerde ik dat onderzoek doen je hele leven leuk kan zijn, even kalm te blijven als het tegenzit en jouw integriteit is voor mij een voorbeeld.

Ik houd van jullie. Zonder jullie was dit niet gelukt.

IV Curriculum Vitae

Emma M.H. Slot was born on November 19, 1992 in Amsterdam, the Netherlands. She completed her secondary education at the Barlaeus Gymnasium (Amsterdam) in 2011. She continued her studies at Amsterdam University College where she obtained her bachelor's honours degree in Liberal Arts and Sciences with distinction (*summa cum laude*).

In 2014 she started her master's degree (Selective Utrecht Medical Master) in Medicine at the University Medical Center Utrecht. During her studies she completed her elective rotation in tropical medicine at Saint Francis Mission Hospital, Katete, Zambia in 2018. She wrote her master's thesis at the department of Child Neurology at the Wilhelmina Children's Hospital in Utrecht.

After graduating in Medicine in September 2018 Emma started working as a resident (ANIOS) at the department of Neurosurgery at the University Medical Center Utrecht. In November 2019 she started her PhD research on the prevention of cerebrospinal fluid leakage after neurosurgery under supervision of dr. T.P.C. van Doormaal and prof. dr. E.W. Hoving at the department of Neurosurgery. As part of her research work Emma was involved in starting and supporting the ENCASE II trial, in which 20 medical centers across the world participate.

Apart from her interest in patientcare and research, Emma pursued her interest in the organization, finance and management of healthcare by joining *Stichting Medical Business* in 2018. She was chair of the national board of *Stichting Medical Business* between 2020 and 2022.

Emma married Jesse Peeters in October 2022. They love to travel together, but are happiest at home in Amsterdam, enjoying the dynamics and diversity of the city.

Emma has started as a resident (ANIOS) in pediatric medicine in January 2023 at Ziekenhuis Gelderse Vallei in Ede.

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Universiteit Utrecht

ISBN 978-94-6483-231-0