Thesis

The Value of β-Trace in CSF-Leakage Detection Confirmed by Endoscopic Na-Fluorescein Evaluation

submitted by

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Graz, 21.09.2024

Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

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Disclosures

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Zusammenfassung

Diese Diplomarbeit beschäftigt sich mit dem diagnostischen Wert des Beta-Trace Protein Nachweises und der intrathekalen Applikation von Natrium-Fluorescein zur Detektion von Liquorfisteln der vorderen Schädelbasis. Als Liquorfistel wird die pathologische Verbindung des mit Liquor gefüllten Subarachnoidalraumes mit angrenzenden Strukturen, im Falle dieser Arbeit der Nasenhaupt- und Nebenhöhlen, bezeichnet. Aufgrund der Seltenheit und Komplexität dieses Krankheitsbildes sowie der hohen Rate an Komplikationen (Meningitiden) ist ein umfangreiches diagnostisches Protokoll zur schnellen Sicherung der Diagnose und genauen Lokalisation der Fistel notwendig. An der Universitätsklinik für Hals-, Nasen- und Ohrenheilkunde der Medizinischen Universität Graz wird hierfür seit Jahren die Kombination aus serologischem Beta-Trace Test, radiologischer Bildgebung (hochauflösende Computertomographie) und präoperativer intrathekaler Applikation von Natrium-Fluorescein verwendet. Ziel dieser Diplomarbeit ist es, die Genauigkeit unseres diagnostischen Protokolls, bestehend aus Beta-Trace Test, in Kombination mit intrathekaler Natrium-Fluoreszein Applikation zur Detektion von Liquorfisteln zu beurteilen. Es wurden die Daten von Patientinnen und Patienten untersucht, welche aufgrund suspizierter Liquorfisteln im Zeitraum von Jänner 2010 bis Dezember 2020 an der Universitätsklinik für Hals-, Nasen- und Ohrenheilkunde der Medizinischen Universität Graz in Behandlung waren. Vor der endoskopischen Operation wurde bei allen Patientinnen und Patienten ein Beta-Trace Test, eine hochauflösende Computertomographie und eine intrathekale Natrium-Fluoreszein Applikation durchgeführt. In dieser Studie wurden Daten von insgesamt 133 Fällen erhoben und nach Anwendung der Einschlusskriterien 28 Personen (9 weiblich, 19 männlich) im Alter zwischen 8 und 69 Jahren inkludiert. Ätiologisch waren die häufigsten Liquorfisteln spontaner Genese (57,1%), gefolgt von traumatischen Fisteln (25%) und iatrogenen Fisteln (17,9%). In 67,8% der Fälle konnte in der hochauflösenden Computertomographie eine knöcherne Dehiszenz an der vorderen Schädelbasis detektiert werden. Bei 75% der Fälle mit positivem Beta-Trace Test wurde die Diagnose der Liquorfistel durch den Fluoresceintest bestätigt und die Fistel im Anschluss endoskopisch verschlossen. Durch die intrathekale Applikation von Natrium-Fluorescein ließ sich in 57,8% der Fälle ein diagnostischer Vorteil gewinnen. Es traten keine perioperativen Komplikationen auf. Die Kombination des Beta-Trace Protein Nachweises und der intrathekalen Applikation von Natrium-Fluorescein hat zuverlässige Ergebnisse

gezeigt. Künftige Forschung sollte sich jedoch auf Alternativen für nicht-invasive Protokolle zur Erkennung von Liquorfisteln konzentrieren.

Abstract

The aim of this thesis is to analyze the diagnostic value of beta-trace protein detection combined with intrathecal sodium-fluorescein evaluation to detect and identify cerebrospinal fluid fistulas of the anterior skull base. A cerebrospinal fluid fistula is defined as a pathological connection between the subarachnoid space (which contains cerebrospinal fluid (CSF)) and adjacent structures, in this thesis the nasal cavity and paranasal sinuses. As a result of the rarity and complexity of this condition as well as the high risk of complications (meningitides), a comprehensive diagnostic protocol is essential for the rapid confirmation and precise localization of fistulas. At the Department of Otolaryngology at the Medical University Graz the combination of testing for beta-trace protein in nasal discharge, radiological imaging (high-resolution computed tomography) and preoperative intrathecal administration of sodium-fluorescein has been the standard diagnostic protocol for many years. This thesis evaluates the accuracy of the beta-trace test in combination with intrathecal sodium-fluorescein administration for detecting cerebrospinal fluid-fistulas. Data was analyzed from patients treated for suspected cerebrospinal fluid fistulas at the Department of Otorhinolaryngology of the Medical University of Graz between January 2010 and December 2020. Before undergoing endoscopic surgery, all patients received a beta-trace test, high-resolution computed tomography and intrathecal sodium-fluorescein administration. For this study, data from a total of 133 cases was collected. After applying the inclusion criteria, 28 patients (9 female, 19 male) between the ages of 8 and 69 years were included. 57.1% of fistulas were of spontaneous origin followed by traumatic fistulas (25%) and iatrogenic fistulas (17.9%). In 67.8% of cases, high-resolution computed tomography detected a bony dehiscence at the anterior skull base. In 75% of cases with a positive beta-trace test, the diagnosis of a CSF fistula was confirmed by the fluorescein test and the fistula was subsequently closed during endoscopic surgery. The intrathecal administration of sodium-fluorescein provided a diagnostic advantage in 57.8% of the cases. No perioperative complications were reported. The combination of testing for beta-trace protein and intrathecal sodium-fluorescein has shown reliable results. However, future research should explore alternatives aiming for non-invasive CSF leak detection protocols.

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List of Abbreviations

CSF	Cerebrospinal fluid
FT	Fluorescein test
HRCT	High-resolution computed tomography
ITF	Intrathecal sodium-fluorescein
ICP	Intracranial pressure
IIH	Idiopathic intercranial hypertension
MRC	Magnet resonance cisternography
MRI	Magnetic resonance imaging
SPSS	Statistical Package for the Social Sciences

1 Introduction

The diagnosis of cerebrospinal fluid (CSF) fistulas and their subsequent precise localization pose a challenging subject within otolaryngology. While the patient's history and clinical examination may lead to the right diagnosis, they are far too imprecise to be used without further diagnostic steps. Typical symptoms of CSF leaks such as clear nasal discharge or headaches are too nonspecific and can be mistaken for other diseases (e.g. allergic rhinitis or sinusitis) or rhinoliquorrhea may go unnoticed due to intermittent or subclinical discharge (2,3).

Several diagnostic tools for the analysis of nasal discharge in suspected CSF leaks have been proposed and tested over the years. Some have been abandoned due to their lack of sensitivity and specificity, such as the glucose oxidase test, others due to their high cost like beta-2 transferrin assays thus leaving beta-trace protein as the marker of choice. However, since beta-trace can only diagnose the presence but not the location of the fistula, further diagnostic steps need to be taken. Radiologic imaging, mainly high-resolution computed tomography (HRCT) may identify the leak site. Nonetheless, even with modern imaging equipment and techniques some leaks may still go unnoticed due to the complex anatomy of the skull base or the presence of multiple or small leaks. If radiologic imaging remains inconclusive despite the presence of rhinoliquorrhea, the fluorescent compound sodium-fluorescein can be utilized to aid the intraoperative search for the CSF leak to ensure its identification and subsequent closure (2,4,5).

A protocol frequently used for the verification of rhinoliquorrhea and its accurate localization is the detection of beta-trace protein within nasal discharge, followed by the intrathecal injection of sodium-fluorescein for an intraoperative endoscopic sodium-fluorescein evaluation (or fluorescein test (FT)) to detect and subsequently close CSF fistulas. While these methods have shown favorable results, no study has examined the value of their joint implementation, especially when considering the potential risks of lumbar puncture and the intrathecal injection of sodium-fluorescein. However, up until the composition and the writing of this thesis the development of standardized diagnostic pathways for the detection of CSF leaks is still ongoing. It is therefore crucial to evaluate the combination of different diagnostic tools as the accurate detection and ensuing repair of CSF fistulas are essential because patients suffering from persisting CSF leaks are at high risk of developing bacterial meningitis. (4,6–10)

To fully appreciate the challenging topic that is CSF fistula detection, the complexity of its etiology as well as the anatomy of the nose and its adjacent structures have to be understood, which is why this thesis will start with the latter.

1.1 Anatomy of the nose and adjacent structures

1.1.1 Nasal cavity

The nasal cavity is located mainly below the anterior cranial fossa, opening anteriorly through the piriform aperture into the nasal vestibulum and posteriorly through the choanae into the nasopharynx. Situated between the anterior cranial base and the hard palate its shape widely resembles a pyramid widening towards the back. The floor is formed ventrally by the premaxilla, the palatine processus of the maxilla and dorsally by the horizontal laminae of the palatine bone. The roof is formed from front to back by the nasal bones, the nasal part of the frontal bone, the lamina cribrosa of the ethmoid bone and the body of the sphenoid. Through numerous small openings (foramina cribrosa) within the lamina cribrosa, fila olfactoria pass into the nasal cavity. These represent endings of the olfactory nerve forming the olfactory epithelium of the nasal mucosa (7,11–13).

The nose is divided sagitally into a left and right nasal cavity by an osseocartilaginous septum. From front to back, it is formed by the pars membranacea, a small part formed by connective tissue behind the tip of the nose (also referred to as “pars mobilis” due to its flexibility), the cartilaginous part (also called lamina quadrangularis) and the bony part formed primarily by the perpendicular plane of the ethmoid anteriorly and the vomer dorsally. To a small extent the nasal bone and body of the sphenoid also take part in forming the bony septum (7,11–13).

The lateral wall of the nasal cavity is formed by the nasal bone, frontal processus and body of the maxilla, the lacrimal bone, the ethmoid with its uncinate processus, middle and superior nasal concha, the inferior nasal concha, and the perpendicular lamina of the palatine bone. The aforementioned conchae form the three nasal meatus situated laterally to the common nasal meatus. Another term, primarily used in clinical practice to describe the structures formed by the nasal conchae, is “turbinates” referring to the underlying bone as well as the overlying mucosa and soft tissue. In rare cases a fourth supreme nasal concha also arising from the ethmoid can be found (11,12).

The inferior nasal meatus is situated between the palatine bone and the inferior nasal concha. Its anterior part contains the nasal aperture of the nasolacrimal duct constricted by an

epithelial fold, called the valve of Hasner. Dorsally, the inferior meatus leads to the opening of the eustachian tube located in the nasopharynx. While the inferior nasal concha is a bone on its own, the middle, as well as superior conchae, are part of the ethmoid. Situated between the two latter structures is the middle nasal meatus. It has great functional importance, as the surrounding paranasal sinuses, i.e. the maxillary and frontal sinus (the latter often indirectly via the ethmoid infundibulum) as well as the anterior and middle ethmoid cells drain into it through the hiatus semilunaris. The structure of the hiatus semilunaris is formed by the posterior edge of the uncinate processus and the anterior part of the ethmoidal bulla, the biggest anterior ethmoid cell. Agger nasi cells can be found frequently, which are located anteriorly to the upper and middle concha. In some cases, the middle concha can be pneumatized by an enclosed air cell and, therefore, enlarged. In this case, it is referred to as concha bullosa. The posterior ethmoidal cells drain into the superior nasal meatus, the space between the middle and superior concha. Located behind the meatus nasi superior, the sphenoid sinus opens into the nasal cavity via the triangular sphenoethmoidal recess (7,11,12,14).

1.1.2 Paranasal sinuses

The paranasal sinuses are part of the pneumatized spaces within the skull. They consist of the frontal, sphenoidal and maxillary sinus as well as the ethmoidal cells. All are located within bones of the same name, draining into the nasal cavity via their ostia. In some cases, accessory sinuses exist, such as an infraorbital ethmoidal cell (Haller cell), an ethmoidal cell anterior to the upper and middle concha (Agger nasi cell), septal cells (when the nasal septum is pneumatized) or a duplicated frontal sinus (sinus frontalis duplex) (12,14).

Development of the paranasal sinuses plays a crucial role during childhood and adolescence by altering the face's shape and size. Although rudimentary paranasal sinuses are present during the fetal period, their development starts after birth, the only exception being the ethmoidal cells and maxillary sinuses, which start their development during the 16^{th} week of pregnancy. Despite being small both, ethmoidal cells and maxillary sinuses, are commonly present at birth. After the second dentition in early adulthood, the maxillary sinuses reach their full extension. Ethmoidal cells start growing rapidly at ages six to eight. The frontal sinuses start their growth at about two years of age after the most anterior ethmoidal cells expand into the frontal bone. At the same time, the most posterior ethmoidal cells extend into the body of the sphenoid forming the sphenoidal sinuses which reach their full size in

adolescence; in some cases, the surrounding walls can be absorbed leading to further growth during adult life (11,12,15).

The sphenoidal sinus lies within the body of the sphenoid bone. It is situated above the nasal choanae and the nasopharynx, constituting the hindmost paranasal sinus. Usually located medially to the superior turbinate on the higher part of the sphenoid body's anterior wall (concha sphenoidalis), the ostia of the left and right sphenoidal sinus open into the nasal cavity via their corresponding sphenoethmoidal recess. Its two irregular and often asymmetrical cavities are separated by an osseous septum, which may be perforated. Furthermore, additional incomplete septa and laminae within each sinus can be found as well (7,11,12).

The expansion of the sinus varies highly between individuals, often transcending the body of the sphenoid. In some cases, particularly large cavities may reach the roots of the pterygoid process, the greater wing of the sphenoid or even the basilar part of the occipital bone. Several classifications for the expansion of the sinus have been introduced over the years, as the pneumatization of the bone has great clinical significance during transsphenoidal skull base surgery. The reason is that the sphenoidal sinus is closely surrounded by numerous vital neurovascular structures, such as the optic nerve (which can be partially encircled), maxillary nerve, oculomotor, trochlear and abducens nerves as well as the cavernous sinus and the internal carotid artery. Extensive pneumatization ultimately leads to thinner osseous walls and therefore greater risk of damaging the aforementioned structures during surgery. Furthermore, dehiscence of the osseous walls of the sinus can be found in some cases, additionally placing these structures at risk as the mucosa of the sinus then is in direct contact with the meninges surrounding the blood vessels and nerves (11,12,16,17).

Unlike other paranasal sinuses, the ethmoidal sinus consists of a multitude of thin-walled cavities called ethmoidal cells. They are located in the upper part of the nasal cavity enclosed medially by the nasal septum and laterally by the wafer-thin lamina papyracea, the division between the nasal cavity and the orbit. Their numbers and sizes vary drastically between individuals, ranging from eight up to 18 cells on each side. Some may not be completely surrounded by bone in which case they are referred to as extramural ethmoid cells (7,11,12). Clinically they are divided into anterior and posterior ethmoidal cells, depending on their embryological development, communication with the nasal cavity, and draining path in relation to the lateral attachment of the middle concha, called the basal lamella. The latter structure separates the anterior from the posterior cells. Front anterior ethmoidal cells drain

into the nasal cavity via the ethmoidal infundibulum, located between the lateral wall of the nose and the ethmoidal uncinate process. However, rear anterior ethmoidal cells drain directly into the middle nasal meatus (7,11,12,18).

One of the most invariable structures is the biggest anterior ethmoidal cell, called the bulla ethmoidalis. Its anterior part forms the hiatus semilunaris along with the posterior edge of the uncinate process, constricting the ostium of the maxillary sinus. In front of the superior and middle concha lie the frontmost ethmoidal cells called agger nasi cells. In about 4% of the population extended pneumatization may involve the orbital floor forming infraorbital or Haller cells in the process (11,12,18).

The posterior cells usually drain into the superior meatus via a single opening. Additional openings into the supreme nasal meatus, if present, or the sphenoidal sinus have also been reported. In about 15% of the population, a posterior sphenoethmoidal or Onodi cell laterally and superiorly to the sphenoidal sinus is present. It is closely associated with the optic nerve, which may even intrude into the cell forming a tuberculum nervi optici leaving the nerve highly vulnerable to damage during endoscopic sinus surgery. (7,11,12).

The frontal sinus is located within the frontal bone. Its shape and size vary drastically between individuals which is why it is regarded as the “fingerprint” of the cranium, useful for identifying individuals in forensic medicine via X-ray. Often asymmetric, it is divided by a similarly off-centered septum into two cavities which may further be divided by additional incomplete septa. A unilateral aplasia can be found in about 10% of the population, while variations of duplicated cavities also exist. The frontal sinus expands into the frontal squama and can pneumatize the roof of the orbit. In most cases, it drains into the nasal cavity, more precisely into the middle meatus, via the frontonasal recess. The frontonasal recess is situated between the lamina papyracea and middle concha of the ethmoid, and ethmoidal infundibulum (7,11,12).

The maxillary sinus is housed within the maxillary bone and resembles the form of a pyramid with its base forming a large part of the lateral wall of the nasal cavity. Its floor, which usually lies lower than the floor of the nasal cavity, is formed by the alveolar process of the maxilla and is in close contact with the roots of the teeth (mainly the second premolar and first molar). It is not uncommon for their radices to be only covered by mucosa due to defects of the overlying bone. The anterior wall corresponds to the facial surface, and the posterior wall to the tuber of the maxilla. Located within the anterior wall is the delicate canalis sinuous which contains the anterior superior alveolar nerve and vessels coming from the infraorbital canal (11,12).

The medial wall houses the ostia of the sinus which are generally located laterally and anteroinferiorly to the uncinate process. The ostia are located further from the floor than the roof; this means that an intact mucociliary escalator is necessary for successful natural drainage. This happens via the ethmoidal infundibulum and the hiatus semilunaris into the middle nasal meatus. The roof is formed by the orbital face of the maxilla and separates the sinus from the orbit while its lateral "tip" extends towards the zygomatic process. The latter can be pneumatized in roughly 40% in which case it is referred to as zygomatic recess. Other recesses within the maxillary sinus are also described, such as an alveolar recess (50%) and less common a palatine or frontal recess (11,12).

1.1.3 Meninges

The central and peripheral nervous system are surrounded by three concentric membranous layers, called meninges. They consist of the dura, the outermost layer, followed by the arachnoid and pia mater. These two are often jointly referred to as "leptomeninges"(11,19). Within the cranium, the opaque and sturdy dura (therefore also called "pachymeninx") firmly merges with the periosteum of the bones surrounding the brain. While it merely follows the brain's contours, leaving out the sulci, its septa (the biggest one being the falx cerebri, separating the two cerebral hemispheres) subdivide the cranial cavity into compartments. Duplications of the dura form the dural venous sinuses, which are responsible for draining venous blood from the brain and meninges (11,12,19).

The much thinner and transparent arachnoid follows the dura's course throughout the nervous system. It surrounds the brain leaving out the sulci and covers blood vessels and cranial nerves. Small arachnoid villi pass through the dura into the venous sinuses and partly into the diploic veins of the skull where they form small pouches called arachnoid granulations or Pacchioni granulations. These are responsible for draining CSF from the subarachnoid space, located between the arachnoid and pia mater, into the venous system (11,19).

The arachnoid is connected to the underlying pia mater via cobweb-like connective tissue fibers (arachnoid trabeculae), hence its name. Unlike the arachnoid and dura, the pia mater closely follows the brain's surface while also covering blood vessels entering the brain right up to the smallest capillaries within the brain. Between the pia mater and the tissue of the brain lies the thin perivascular space or Virchow-Robin space (11,19).

1.2 Cerebrospinal fluid

Approximately 500 ml of CSF, a clear colorless liquid, are produced daily by the choroid plexus, mainly in the lateral ventricles and, to a lesser extent, by the ependyma of the ventricles and the spinal cord. CSF is actively secreted and modified by the plexus epithelium, therefore not solely being an ultrafiltrate of the blood, the latter containing much higher amounts of cells, proteins, and glucose. Its production follows a circadian rhythm, with a 3.5 higher secretion rate at 2:00 AM as opposed to 6:00 PM. Taking into consideration that the total volume of CSF in adults is about 150 ml means that the entirety of CSF is replaced three to four times each day. This overproduction could explain why CSF leaks, depending on their size and drainage, can remain asymptomatic for months or even years (11,19,20).

After being secreted in the lateral ventricles, CSF flows through the interventricular foramen of Monro into the third ventricle and then via the aqueduct of Silvyus into the fourth ventricle in the area of the brain stem. Communication with the cisterna magna, located between the cerebellum and medulla oblongata, is made possible by the central foramen of Magendie and the two lateral foramina of Luschka. From there on the flow continues *"dorsally into the subarachnoid space of the cerebellum, caudally into the spinal subarachnoid space, and rostrally into several subarachnoid cisterns of the brain."*(20) where CSF reaches the subarachnoid space of the cerebral hemispheres. Arterial branches of the circle of Willis lie within the subarachnoid space of the major cisterns, resulting in vascular pulsation of CSF (19,20).

Within the cranium, CSF is resorbed into the brain's venous system, the sinus durae matris, through the arachnoid villi and Pacchioni granulations. In the spinal cord and skull base area veins and lymphatic vessels surrounding the exit points of spinal and cranial nerves receive the CSF. Absorption is mainly determined by hydrostatic pressure within the subarachnoid space and pressure within the venous system (19,20).

CSF functions as a fluid cushion distributing pressure from outside trauma keeping the brain safe while simultaneously reducing its actual weight from 1400g to roughly 50g. Furthermore, CSF is responsible for maintaining a consistent extracellular milieu, removing potential harmful metabolites, and regulating neural functions of the respiratory center in the medulla oblongata by mediating CO_2 plasma concentrations. Another important task of CSF is to protect the brain from changes in intracranial pressure (ICP) due to fluctuations in intracranial blood flow. Normally, ICP ranges from five to 15 mmHg. Prolonged

pathological increases in ICP can occur post-traumatically, after obstruction of CSF drainage (e.g. due to tumors), or, on rare occasions, due to vast increases in CSF secretion (e.g. due to choroid plexus carcinoma) which may result in structural damage of the brain. This can range from cranial or spinal CSF leaks up to herniation of the brain through the falx cerebri, tentorium cerebelli, or the foramen magnum, the latter being life-threatening conditions if not treated promptly (19–21).

1.3 Cerebrospinal fluid fistulas

1.3.1 Definition

Cerebrospinal fluid fistulas occur when a pathological connection between the nasal cavity or paranasal sinuses and subarachnoid space is formed resulting in watery nasal discharge. For this to happen a total breach of the leptomeninges and dura mater as well as bone and nasal mucosa has to occur. On rare occasions a communication to the middle ear is responsible, however 91% of CSF fistulas are caused by frontobasal defects which is why this thesis focuses primarily on the latter. While showing the same symptoms, the former is referred to as oto(rhino)liquorrhea as opposed to rhinoliquorrhea (7,22).

1.3.2 Etiology

The most common cause for CSF leaks is head trauma, responsible for 80% of craniobasal CSF fistulas. Research shows these occur in about 1-3% of closed head traumas (7). CSF leaks resulting from endoscopic or neurosurgical operations make up 16%, as iatrogenic injuries happen in approximately 10% of skull base surgeries. Structures at risk are e.g. the lamina cribrosa or fovea of the ethmoidal bone, the frontal sinus or the sphenoidal sinus. Another factor contributing to this form of CSF leaks could be a temporary postoperative increase in ICP brought on by reduced resorption of CSF due to irritation of the arachnoid granulations (3,13).

About 3-4% of cases consist of primary CSF leaks i.e. where discharge happens spontaneously. This etiology is by far the rarest and probably least understood, however, over the last years many studies have pointed towards an association with elevated ICP (2,3,23–25). This evidence becomes especially clear when comparing the epidemiology of patients diagnosed with spontaneous CSF leakage or idiopathic intracranial hypertension (IIH). Both groups share a very similar population, namely middle-aged overweight women, as well as typical radiological signs such as empty sella, arachnoid pits, changes of the optic

sheath complex or thinning of the skull base (2,23,26–28). Barañano et al. (29) found an almost five times higher incidence of arachnoid pits in CT scans within this patient collective. A theory why this could be the case involves the interaction of higher body fat and extraovarian production of estrone within fatty tissue. This might lead to relative hypoadrenalism resulting in higher resistance to CSF outflow because of altered drainage channels and vacuolar transport across arachnoid granulations (29).

Newer studies seem to show a change regarding relative frequency of CSF fistula etiologies, shifting the order of causes to spontaneous followed by traumatic and iatrogenic from most to least frequent. As to why, the cause for this change might be safer vehicles or mandatory crash helmets for cyclists and bikers, more operation expertise and heightened awareness of spontaneous CSF leaks (30,31).

Other pathologies leading to an increase in ICP like pseudotumor cerebri, slow-growing tumors obstructing CSF drainage or hydrocephalus also present risk factors for CSF leaks. As stated by Dunn et al. (26), in these cases the defect acts as a safety valve to reduce ICP, which could explain why patients commonly do not show typical symptoms of increased ICP (7,26,28).

Malformations of the cranial base, especially the body of the sphenoid bone, might also play a role; however, whether leaks in these locations arise on their own or in conjunction with increased ICP remains a topic of discussion (7,32,33).

1.3.3 Symptoms

The main presentation of CSF fistulas is clear watery discharge from the nose, most commonly unilaterally and intermittently. Depending on the head's position fluid might also drain via the posterior nasal aperture and nasopharynx resulting in some patients describing a sweet or salty taste. Also dependent on head positioning is the amount of drainage dubbed as "reservoir sign"(3).

As stated above patients with pathologies resulting in increased ICP lack the classical symptoms and clinical features of increased ICP i.e. papilledema leading to visual problems which present in over 90% of patients suffering from IIH. This leads to the risk of elevated ICP going unnoticed and manifesting itself only after surgical closure of the leak (28).

It is important to note that symptoms in patients with CSF leakage resulting from head trauma can manifest after a latency period of a few weeks up to several years. This could be due to contraction or necrosis of tissue surrounding the defect, regression of edema or an increase in ICP (3).

1.3.4 Diagnostics

1.3.4.1 Patient history and clinical examination

While the diagnostic pathway of CSF leak detection poses a challenging topic with lots of different diagnostic tools, it is important to start each patient encounter by taking a thorough patient history combined with a detailed clinical examination. Patients with CSF leaks typically report intermittent (and mostly unilateral) clear nasal discharge. Some may describe a sweet or salty taste, resulting from CSF drainage via the nasopharynx, however, the "International Consensus Statement: Spontaneous Cerebrospinal Fluid Rhinorrhea" (2) deemed this manifestation as too unspecific. It is also important to note that small amounts of CSF leakage may go unnoticed by the patient. In addition, it should be kept in mind that serous nasal discharge due to infections of the nose or paranasal sinuses (most commonly the maxillary sinus) can present themselves in a very similar way to rhinoliquorrhea. Therefore, it is vital to assess other symptoms as well as the patient's past medical history. As mentioned above CSF leaks due to injuries of the skull base can first present themselves weeks or even months after the initial trauma. On the other hand, patients with acute head trauma can temporarily show signs of CSF leakage due to damage of the dura surrounding the ethmoid's fila olfactoria even if no fracture is present (and, therefore, duraplasty should not be performed). However, as a general rule suspected defects of the skull base should always be further examined (2,3,7).

Less common etiologies of CSF leaks may present themselves in different ways. For example, patients with congenital malformations or defects of the skull base may report recurrent meningitides often caused by Streptococcus pneumoniae (7).

Though the rarest cause for rhinoliquorrhea, it is also important for the examiner to know the risk profile of patients with IIH and their symptoms. These include frequent headaches, pulsatile tinnitus, and visual defects due to papilledema. As stated above and examined in studies (28,34) the prime collective of patients diagnosed with IIH are middle-aged obese women. However, it has also been established that IIH patients with CSF leaks tend to not develop papilledema and, therefore, do not show signs of visual impairment or any symptoms typically associated with IIH. Nevertheless, radiological signs of increased ICP can be present and are listed below (2,28).

1.3.4.2 Radiologic Imaging

The role of radiologic imaging in CSF leakage detection remains a controversial topic. In clinical practice high-resolution computed tomography (HRCT) and magnetic resonance imaging (MRI) have established themselves as the methods of choice. While HCRT has the edge over MRI concerning cost and availability, it has earned its fair share of criticism; for one, due to patients' exposure to radiation and its inability to detect small leaks, bony dehiscences or non-dislocated fractures respectively, even when clinical and biochemical findings strongly suggest a CSF leak. Furthermore, due to its imaging technique evaluation of the leptomeninges is not possible. Therefore, HRCT (high-resolution computer tomography) is only able to show indirect signs of CSF fistulas such as fractures or erosions of the skull base, intracranial air or meningoencephaloceles. As a consequence, it is important for radiologists to know what to look for and where. Le et al. (3) have stated that leaks due to trauma *"[...] most commonly occur in the sphenoid sinus (30%), frontal sinus (30%), and cribriform plate/fovea ethmoidalis (23%)"*(3).

On the other hand, MRI is able to show the structure of the leptomeninges and, therefore, its pathologies (e.g. leaks) but its implementation into the diagnostic protocol of CSF leakage detection remains severely limited due to high cost and sparse availability. However, in cases of suspected CSF leaks with no apparent cause present, MRI can show signs of IIH which include *"[...] empty sella, abnormalities of the optic sheath complex, globe flattening, encephaloceles, arachnoid pits, enlarged Meckel's cave, and dural ectasia"*(2). In addition, it is important to keep in mind that patients with spontaneous CSF leakage and IIH may have multiple leaks or defects of the skull base (4,7,35,36).

Newer studies indicate favorable results regarding the diagnostic use of magnet resonance cisternography (MRC), showing even better results than HRCT. This noninvasive imaging technique is able to confirm and localize leaks by highlighting the bright signal of CSF in T2-weighted images. It is possible to combine this method with intrathecal gadolinium-based contrast media. While this approach further enhances the diagnostic value of MRC, the downsides of potential risks associated with intrathecal injection, the use of contrast agents as well as high costs have to be kept in mind (10,37).

Other imaging modes such as head X-rays or CT-cisternography are no longer being used due to their limited diagnostic value and the latter also due to its invasiveness and use of intrathecal contrast media (4,7,36).

1.3.4.3 Beta-trace protein

Beta-trace or prostaglandin D synthase is a small secretory protein exclusively produced within the brain, mainly by the leptomeninges. Under physiological conditions, it follows a steep rostrocaudal gradient, leading to an increase in concentration along its flowing path towards the spinal cord. Over the last years it has gained an important role as a marker for liquorrhea due to its abundance in CSF, exceeding the blood concentration by a factor of 34 (4,38).

Nasal discharge is tested using a nephelometer, which is an immunologic assay utilizing antigen-antibody complexes to scatter light. This method provides major benefits including its fast measuring and processing time (results are ready within 20 minutes), very high sensitivity and specificity, as well as low cost. Furthermore, relatively small fluid samples are required, 200 µl being enough for one measurement, which can be obtained non-invasively. Despite its many advantages the statement *"All patients suspected of CSF rhinorrhea should have the nasal fluid examined for beta trace protein or, if not available, for beta transferrin"*(2) published in the 2021 "International Consensus Statement: Spontaneous Cerebrospinal Fluid Rhinorrhea" did not receive universal agreement. One reason being that testing for beta-trace was not readily available in parts of the United States. Furthermore, some of the authors argued that testing for beta-trace is redundant when radiological signs as well as clinical presentation strongly hint at CSF rhinorrhea, examining nasal fluid only if *"no obvious identifiable defect and/or mechanism"*(2) for CSF leakage was found. This led to the statement being changed to *"Examination of the nasal fluid for beta2 transferrin/beta trace protein is an option if there is no obvious identifiable defect and/or mechanism of CSF leak."*(2) (2,4,9,39,40).

One huge disadvantage in the detection of beta-trace, especially when comparing results from other publications and clinics, are non-uniform cutoff values, probably due to differences in study design. In 2005 Risch et al. (41) suggested a cutoff value of 1.11 mg/l to detect patients with CSF leakage, which was later confirmed by other studies (4). Concentrations between 0.68 to 1.10 mg/l are the most problematic and may require a comparison of the concentrations within serum and nasal discharge. If the ratio is above 4.9 CSF leakage is highly likely, while ratios of <1 exclude CSF leakage (4,7,41).

Other factors that have to be considered are the effects of renal insufficiency and bacterial meningitis on beta-trace protein levels. Tumani et al. (42) have shown a significant decrease in beta-trace protein concentration in patients suffering from bacterial meningitis, even after

successful treatment, normalization of CSF leukocytes and the function of the blood-brain barrier. In addition, it is important to examine the patient's renal function when blood beta-trace protein has to be measured (e.g. when nasal concentrations are inconclusive) as renal insufficiency leads to increased levels of beta-trace in the blood (4,42).

1.3.4.4 Beta-2 transferrin

Beta-2 transferrin is one of seven desialated transferrin isoforms found almost exclusively within CSF. In 1979 Meruman et al. (43) first described its use for CSF leakage detection stating that *"[...] this β2-transferrin is pathognomonic for liquor and could not be demonstrated in serum, nasal secretions, saliva, tears, or peri-and endolymph."*(43). Further studies have shown that this statement is only partially correct as low concentrations of beta-2 transferrin can be found within the inner ear's perilymph and the eye's vitreous and aqueous humors (7,35,44–46).

Nevertheless, sensitivity and specificity as well as positive and negative predictive values are excellent. False positive findings are possible resulting from elevated systemic levels of beta-2 transferrin due to impaired liver function (e.g. cirrhosis) or infections with bacteria secreting sialidases, which turn sialated transferrin in nasal mucus into its desialated form (4,47).

Similar to beta-trace, nasal discharge is collected non-invasively from patients with suspected CSF fistulas. Even though samples as low as 10 µl are enough for one measurement, higher volumes (usually two to five ml) are necessary to achieve the desired diagnostic performance; this could lead to problems in cases with intermittent or low-flow leaks resulting in a delayed diagnosis. How protein degradation due to longer collection times could lead to false-negative results remains unknown. However, the biggest drawback regarding beta-2 transferrin arises during laboratory testing. Its detection is most commonly achieved by immunofixation electrophoresis, an expensive as well as labor-intensive process with hands-on time between two and four hours, further delaying diagnoses. This is why beta-2 transferrin has been universally replaced by beta-trace protein in routine clinical practice (4,7,40,41,47).

1.3.4.5 Glucose oxidase test

Measuring glucose levels within nasal discharge is a traditional way of diagnosing CSF leaks. This is possible due to differences in the composition of CSF compared to other bodily fluids. Although this test is readily available, cheap, fast, and easy to perform it lacks

diagnostic sensitivity and specificity (false-negative results in case of bacterial contamination, false-positives with hyperglycemic patients) which has led to it being replaced by other more reliable detection methods (4).

1.3.4.6 Sodium-fluorescein

The potential use of intrathecal sodium-fluorescein (ITF) was first described in 1960 by Kirchner and Proud who concluded that *"[...] fluorescein injection intraspinally and searched for under UV light is of practical value in the identification and localization of CSF leakage."*(48).

Since then the use of ITF has been widely implemented into the diagnostic protocol for the identification and localization of CSF fistulas, especially when radiologic imaging has been inconclusive despite the presence of rhinoliquorrhea. The yellow-greenish fluorescent compound is administered intrathecally via lumbar puncture hours before the operation, giving it time to spread throughout the CSF. As the latter is a colorless fluid and hard to spot during surgery, the physical and chemical properties of sodium-fluorescein come in very useful as it appears bright green-yellowish when viewed at under UV light. This means that false-positive results are not possible, while false-negative findings happen in roughly 1-7% of cases. In 2010 Seth et al. (49) stated that false-negative results occur in up to 26% of cases (3,4,7,49–53).

The fact that intrathecal injection via lumbar puncture is necessary to utilize the properties of sodium-fluorescein has raised many questions regarding its risks and potential side effects. For one, sodium-fluorescein cannot be administered if contraindications for lumbar punctures (e.g. elevated ICP, purulent meningoencephalitis with impaired awareness or acute severe traumatic brain injury) are present. Moreover, adverse reactions such as seizures, limb weakness, coma, or even death have been documented (53). Nevertheless, it is crucial to note that these complications are primarily associated with high doses exceeding 50 mg. Lower doses of ITF on the other hand are considered generally safe, while their diagnostic value remains unimpaired (3,52). However, as Jolly et al. (53) pointed out, no internationally accepted dosage has been agreed upon. Ultimately, even though ITF has played an important role in the detection of CSF fistulas for many years, its use remains mostly off-label due to lacking approval or licensing issues in many countries (3,7,50–53).

Another potential approach regarding sodium-fluorescein is intraoperative topical application. While this method is simple to perform, inexpensive and eliminates intrathecal

injection with its associated risks and side effects, its use remains fairly scarce resulting in limited study data with varying results (5,54).

1.3.5 Complications

The fact that leaks may go unnoticed as the discharge can be minimal or might drain via the nasopharynx presents a problem as persisting CSF fistulas come with a high risk of bacterial meningitis. This is especially relevant for patients with traumatic CSF leaks, who have a higher risk of developing bacterial meningitis compared to patients with other etiologies. According to Bernal-Sprekelsen et al. (8), up to 33% of patients with posttraumatic CSF leaks might develop bacterial meningitis, other authors state a risk of 9-50% while the overall risk over a ten-year period is estimated at 85% (6–8).

Patients with primary leaks have a noticeably lower risk of developing bacterial brain infections. This might be caused by elevated ICP, resulting in high-pressure leaks, making it harder for bacteria to ascend and infect the meninges or brain (23).

While thin arachnoid-scars can form over the defects causing the discharge to stop, this does not seem to reduce the risk of meningitis as these thin layers provide inadequate protection against bacterial ascension. However, it is important to note that antibiotic prophylaxis is not recommended as it has not shown favorable results. This means that endoscopic closure of the defect seems the best prophylactic measure against infections while also being the treatment of choice for CSF leaks (7,55).

1.3.6 Therapy

As stated above the primary goal of therapy is to close the defect responsible for CSF leakage. A verified CSF fistula with persisting or intermittent nasal discharge presents the indication for closure of the defect or duraplasty. It has been agreed upon that the procedure should be done as soon as possible, as there is no substitute for this operation or place for conservative measures (2).

Over the last years endoscopic CSF leak repair has been established as the therapy of choice with success rates well above 90% as well as low perioperative morbidity and mortality. Extranasal approaches are rare and only used when the leak cannot be reached via the nasal cavity e.g. when situated in the lateral frontal sinus (3,7).

Before operative closure, it is mandatory to verify the leak and identify its location. Regardless of which approach is being used, the basic procedure stays the same starting with the excision of the meningoencephalocele, if present, followed by preparation and excision

of the mucosa around the leak. Many techniques and materials, including autologous grafts such as fascia lata, mucosal flaps, temporal fascia, and ear or septal cartilage are available for covering the defect (2,56).

When the underlay technique is used the dura must first be detached from the bone for the graft to be inserted. It can either be placed between the arachnoid and dura (intradural underlay) or between the dura and bone (extradural underlay) and must be placed in such a way that it covers the leak on all sides. If there is a risk of damaging neurovascular structures lying beneath the bone while dissecting or the underlay method simply is not possible, the onlay (also called overlay) method is used. Here the graft is placed over the skull base defect. Whichever method is used, the goal is for the graft to form a tight seal consisting of scar tissue as a result of wound healing. A lumbar spinal drain can be placed during the procedure, subsequently lowering the CSF pressure. This has been recommended in many studies, although it is not always necessary. However, if the underlying condition leading to CSF leakage is the result of increased ICP or IIH, it is mandatory to treat patients accordingly i.e. ICP lowering measures. Otherwise, they might develop symptoms of high ICP (as stated above, CSF leaks may act as safety valves to compensate for the increased ICP and patients typically do not show symptoms of elevated ICP) or recurrent CSF leaks (2,7,22,26).

1.4 Aim and hypothesis

The detection of beta-trace protein in nasal discharge and the subsequent intrathecal sodium-fluorescein application are frequently used in conjunction by the Department of Otolaryngology at the Medical University Graz for the diagnosis and precise localization of CSF leaks. This study's hypothesis was that the combination of beta-trace protein testing, HRCT and ITF evaluation would provide a reliable protocol for confirming and localizing CSF leaks. Therefore, this thesis aims to assess the use of beta-trace detection and subsequent intrathecal sodium-fluorescein application to detect and localize CSF leaks in conjunction with HRCT imaging.

2 Methods

2.1 Setting

This thesis was conducted monocentric at the Department of Otorhinolaryngology at the Medical University of Graz.

2.2 Study Design

Patient data was obtained retrospectively via electronical hospital records of the Department of Otolaryngology of the Medical University of Graz. The first step was preselecting eligible cases which was performed by the Institute for Medical Informatics, Statistics, and Documentation by searching for specific operation service codes in the period from January 2010 to January 2021. This resulted in a total of 133 cases spanning from January 2010 to December 2020.

2.2.1 Inclusion criteria

- Patients who had a beta-trace test done and underwent intrathecal sodium-fluorescein injection followed by nasal endoscopic surgery
- Ages between 5 and 85
- Male and female
- Diagnosis of liquorrhea

2.2.2 Data collection

Data was collected retrospectively by examining electronical hospital records documented in the institutional Medical Documentation and Communication system. Patients' data (i.e. names, dates of birth, and ID numbers) were moved to an encrypted Microsoft-Excel© spreadsheet and subsequently converted and imported into the IBM© Statistical Package for the Social Sciences (SPSS) for data analysis. The analyzed patient parameters are listed in Table 1. The accuracy and reliability of the collected medical data was carefully assessed. Individuals who did not meet the inclusion criteria were excluded from the study.

Table 1: Assessed patient parameters

Parameter	Value
Age	years
Sex	female; male
Height	centimeters
Weight	kilograms
BMI	kilograms per square meter
Diagnosed OSAS (obstructive sleep apnea syndrome)	yes; no
Cardiovascular risk factors	yes; no
Liquorrhea duration till hospital visit	days
Liquorrhea duration till operation	days
Follow-up period	months
Duration of hospitalization	days
Etiology of liquorrhea	spontaneous; traumatic; iatrogenic
Iatrogenic cause	uncomplicated FESS (functional endoscopic sinus surgery); skull-base tumor operation
If iatrogenic cause: Indication for operation	hearing impairment; chronic sinusitis; septum deviation; olfactory groove meningioma
Diagnosed IIH	yes; no
If diagnosed IIH: Symptoms present	yes; no
Diagnosed elevated ICP	yes; no
Diagnosed sinusitis	yes; no
Diagnosed polyposis	yes; no
Performed HRCT scan	yes; no
If HRCT scan performed: Meningoencephalocele visible	yes; no
If HRCT scan performed: Leak visible	yes; no
Performed MRI scan	yes; no
If MRI scan performed: Typical signs of IIH	yes; no

Diagnosed meningitis	yes; no
Antibiotics administered	yes; no
Beta-trace or beta-transferrin test done	yes; no
If beta-trace or beta-transferrin test done: Beta-trace	yes; no
If beta-trace or beta-transferrin test done: Beta-transferrin	yes; no
If beta-trace or beta-transferrin test done: Test repetitions	n
If beta-trace test done: Result	milligrams per liter; results above 1.69 mg/l were considered “positive”
If beta-transferrin test done: Result	milligrams per deciliter; positive
Sodium-fluorescein administered	yes; no
If sodium-fluorescein administered: Volume	milliliters
Duration between sodium-fluorescein administration and operation	hours
If sodium-fluorescein administered: Complications	yes; no
If sodium-fluorescein administered: Visible fluorescence during operation	yes; no
Visible liquorrhea during operation	yes; no
Intraoperative lumbar drainage performed	yes; no
Material used for closure of CSF fistula	free mucosa flap; pedicled flap
Visible leakage after closure	yes; no
Surgical complications	yes; no
If surgical complications: Intra- or postoperative?	intraoperative; postoperative
If surgical complications: Type	description
Tamponade used	yes; no
If tamponade used: Type	Rhinorapid; Tabotamp; GELITA sponge; Nasopore
Tamponade dwell time	days

Antibiotics administered perioperatively	yes; no
If antibiotics administered perioperatively: Duration	days
Recurrence of liquorrhea	yes; no
If recurrence of liquorrhea: Time until recurrence	days

2.3 Diagnostic protocol

At the Department of Otorhinolaryngology of the Medical University of Graz the diagnostic protocol for suspected CSF leaks starts with taking the patients' history, asking for specific symptoms (see above) and conducting a routine clinical examination. Next, nasal discharge is tested for beta trace protein to confirm the presence of CSF. For this a small sample of nasal fluid has to be collected. Results above 1.69 mg/l are considered "positive", confirming rhinoliquorrhea, while negative results may require further examination if the clinical suspicion remains high.

The following step involves radiologic imaging, the method of choice being HRCT of the skull base and paranasal sinuses to identify direct or indirect signs of CSF leaks (see above). While not part of the routine protocol, an additional MRI can be performed to further evaluate soft tissue structures, especially if the HRCT's results were inconclusive. If the analysis of nasal fluid confirms the presence of CSF and/or if radiologic imaging shows signs of CSF leaks, the planning and scheduling of the surgery have to be undertaken. Patients are informed about the endoscopic operation, the procedure, its objectives and its necessity before giving consent.

Sodium-fluorescein is administered intrathecally via lumbar puncture the afternoon or evening before the surgery for precise intraoperative localization of the leak. CSF drawn is usually sent to the laboratory for analysis.

During the endoscopic surgery, the fluorescent properties of sodium-fluorescein are used to precisely localize the leak. If no fluorescence is visible, the preoperative radiologic imaging is utilized to approximate the leak's location. Afterwards the leak is closed employing appropriate techniques and materials (see above) to stop CSF drainage and form a tight seal. The closure is then checked for any additional leaks to ensure its effectiveness.

In some cases, an intraoperative lumbar drain is performed to reduce intracranial pressure and aid the healing process. A perioperative antibiotic prophylaxis may be administered as well to prevent postoperative infections.

Finally, the use of nasal tamponades after surgery might be required to support the surgical site and ensure a successful closure of the defect. This comprehensive protocol ensures an accurate diagnosis and effective treatment of CSF leaks.

2.4 Primary variables of interest

The primary variables of interest were the diagnostic accuracy regarding the combination of testing for beta-trace, HRCT imaging and the use of intrathecal sodium-fluorescein for detecting and localizing CSF leaks in the skull base.

2.5 Statistical Analysis

For the statistical analysis the IBM© Statistical Package for the Social Sciences (SPSS) for data analysis was used. Continuous parameters are described using the mean and standard deviation, categorial parameters by their absolute numbers and relative percentages.

2.6 Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Medical University of Graz for studies involving humans. Due to the retrospective nature of this thesis the necessity for written informed consent was waved by the Institutional Review Board of the Medical University of Graz. Only the author and supervisor had access to patient data, which was stored in an encrypted Excel spreadsheet. All patients were given numbers, which were then imported into SPSS leaving only anonymous data for further statistical analysis.

3 Results

Carefully examining the data provided by electronic hospital records for the determined parameters have led to the following results: one case was excluded due to its complexity and missing vital study data, two cases were duplicates and 102 did not meet the inclusion criteria leaving a total of 28 cases for analysis.

3.1 Clinical characteristics

3.1.1 Demographic data

This study included a total of 28 patients, 9 females (32.1%) and 19 males (67.9%). The youngest patient was 8 years of age, the oldest 69 years while the mean age within this patient collective was 47.5 years ± 16.2 years. The mean BMI was 28.8 kg/m^2 ± 6.3 kg/m^2 spanning from 15.2 kg/m^2 to 44.1 kg/m^2.

3.1.2 Comorbidities

Within this patient collective 3 (10.7%) were diagnosed with OSAS, 22 (78.6%) had cardiovascular risk factors (e.g. elevated blood pressure, dyslipidemia, or obesity), none (0.0%) were diagnosed with IIH, 2 (7.1%) patients had diagnosed elevated ICP, 11 (39.3%) had diagnosed sinusitis, 4 (14.3%) had diagnosed polyposis and 2 (7.1%) had diagnosed meningitis.

3.1.3 Liquorrhea durations

The average time of liquorrhea until patients visited the hospital was 137.4 days ± 550.5 days, spanning from 0 days up to 2920 days. Average time from first manifestation of CSF leaks until the operation was 143.8 days ± 554.5 days.

3.1.4 Antibiotics

23 (82.1%) patients received perioperative antibiosis. The average duration of antibiotic administration during hospital visits was 8.1 days ± 3.4 days, ranging from 5 days up to 20 days. 10 (35.7%) patients had received antibiotic treatment prior to their hospitalization.

3.2 CSF leak etiologies

7 (25.0%) leaks were caused by trauma, 5 (17.9%) were complications resulting from operations (4 (80.0%) during functional endoscopic sinus surgery and 1 (20.0%) during skull-base tumor surgery) and 16 (57.1%) cases were categorized as “spontaneous” as no clear causes could be found.

3.3 Perioperative management

In 17 (60.7%) cases liquorrhea was observed before using UV-light. 22 (78.5%) patients showed clear signs (either visible with the naked eye or after positive IF-evaluation) of present CSF fistulas, of which 20 (91.0%) were closed using a free mucosa flap and 2 (9.0%) using a pedicled flap. Closure upon first attempt was achieved in 15 (68.1%) cases. An intraoperative lumbar puncture was performed in 3 (10.7%) cases.

There were no intra- or postoperative complications (0.0%). 3 (1,1%) patients received an intraoperative lumbar drain.

3.3.1 Tamponade usage

After surgery tamponades were placed in 16 (57.1%) cases. Tamponade types included Rhinorapid in 10 (62.5%) cases, Tabotamp in 4 cases (25.0%), Nasopore in 1 (6.25%) case as well as GELITA sponge in 1 (6.25%) case. The average dwell time was 3.3 days ± 2.1 days, ranging from 1 day up to 7 days.

3.3.2 Recurrences

In total 7 (25.0%) patients had a CSF leak recurrence. The shortest time until the recurrence was 1 day, the longest 208 days averaging 44.1 days ± 74.6 days.

3.3.3 Duration of hospitalization and follow-up period

Hospitalization of this patient collective ranged from 2 days up to 53 days with an average duration of 18 days ± 15.5. The follow up period was 14.2 days ± 10 days on average ranging from 9 days to 32 days.

3.4 Primary variables of interest

3.4.1 Radiologic imaging

In each case an HRCT scan was performed. Leaks were visible in 19 (67.9%) cases. A meningoencephalocele was visible in 1 (3.7%) CT-scan.

MRIs were performed in 11 cases (39.3%) of which none (0.0%) showed typical radiological signs of IIH.

3.4.2 Beta-trace

In 26 (92.8%) cases testing for beta-trace was performed once, in 1 case testing was repeated once (3.6%) and in another case (3.6%) a total of three tests were performed. 8 (28.6%) results were merely labeled as "positive" while results of the other 20 (71.4%) cases showed an average concentration of 20.5 mg/l ± 2.7 mg/l. In total 22 (78.5%) patients had confirmed CSF fistulas. Overall, the sensitivity of beta-trace, accurately predicting later confirmed CSF leaks, was 78.5%.

3.4.3 Sodium-fluorescein

On average 0.7 ml ± 0.2 ml of sodium-fluorescein was administered intrathecally, ranging from 0.3 ml up to 1 ml. The average duration between administration and operation was 15.2 hours ± 5.2 hours, the minimum time being 0.5 hours and the maximum time 33 hours.

During the operation in 21 cases (75.0%) visible fluorescence could be observed, meaning the FT was positive. One patient (3.6%) showed no visible fluorescence despite an apparent CSF leak with visible CSF flow. In 5 cases (17.9%) no CSF flow could be observed before using UV light to detect the administered ITF.

There were no complications associated with ITF in all 28 cases (100.0%).

Table 2: Diagnostic details of assessed cases

	Beta-Trace	Leak in HRCT	ITF	Cause
Case 1	Positive	Seen	Positive	Spontaneous
Case 2	Positive	Seen	Positive	Spontaneous
Case 3	Positive	Seen	Positive	Spontaneous
Case 4	Positive	Not seen	Positive	Iatrogenic
Case 5	Positive	Seen	Positive	Trauma
Case 6	Positive	Seen	Positive	Spontaneous
Case 7	Positive	Seen	Positive	Spontaneous
Case 8	Positive	Seen	Positive	Trauma
Case 9	Positive	Seen	Positive	Spontaneous
Case 10	Positive	Not seen	Negative	Spontaneous
Case 11	Positive	Not seen	Positive	Trauma
Case 12	Positive	Seen	Positive	Spontaneous
Case 13	Positive	Seen	Negative	Spontaneous
Case 14	Positive	Seen	Positive	Iatrogenic
Case 15	Positive	Not seen	Positive	Trauma
Case 16	Positive	Seen	Positive	Iatrogenic
Case 17	Positive	Seen	Positive	Spontaneous
Case 18	Positive	Seen	Positive	Spontaneous
Case 19	Positive	Seen	Positive	Spontaneous
Case 20	Positive	Not seen	Positive	Trauma
Case 21	Positive	Not seen	Negative	Spontaneous
Case 22	Positive	Seen	Positive	Trauma
Case 23	Positive	Seen	Positive	Spontaneous
Case 24	Positive	Seen	Positive	Iatrogenic
Case 25	Positive	Seen	Negative	Trauma
Case 26	Positive	Not seen	Negative	Spontaneous
Case 27	Positive	Not seen	Negative	Iatrogenic
Case 28	Positive	Not seen	Negative	Spontaneous

4 Discussion

Patient characteristics of this thesis correspond largely with published data. Psaltis et al. (31) reported an average age of 47.2 years based on the analysis of 44 studies, almost matching the average age of 47.5 years in this study. And while their age span ranged from 27 to 61 years, this study includes patients from 8 to 69 years. Differences also arise with gender distribution as their results pointed to *"an almost equal gender distribution"*(31) whereas this study includes 32.1% female and 67.9% male subjects. However, Felisati et al. (51) as well as Keerl at al. (52) observed a higher number of male patients with 67% males and 33% females in the former and 58.5% males and 41.5% females in the latter, thereby more closely resembling the results of this study. Keerl et al.'s (52) average age of 46.9 years (spanning from 1 year up to 82 years) also closely resembled the results of this study.

The overall percentage of patients diagnosed with meningitis prior to surgery in this study is 7.1%. Comparing this result with studies focusing on the relationship of meningitis and CSF fistulas this number is lower than expected. In the systematic review conducted by Psaltis et al. (31) 23.5% of the patients' collective had episodes of meningitis before surgery, Dunn et al. (26) reported meningitis rates of 26.6%. Poletti-Muringaseril et al. (23) focused on the differences of meningitis rates depending on the CSF leak being primary or secondary and found that primary CSF leaks were less likely to cause meningitis with 17% of patients reporting a history of meningitis as opposed to 29% of patients suffering from secondary CSF leaks.

In this study 25% of leaks were caused by trauma, 17.9% due to iatrogenic injury during previous skull base operations and 57.1% of leaks happened spontaneously. Historically, traumatic injury was considered to be the most common cause of CSF leaks, literature stating rates of up to 80% while spontaneous leaks were deemed much rarer, well within the singular percentage range (4,7,13).

However, recent studies indicate a shift in the relative frequency of CSF fistula etiologies, with spontaneous causes now being the most common type, followed by traumatic and iatrogenic causes in descending order of frequency. As to why, studies suggest better diagnostic tools, a better recognition of the spontaneous etiology and a shift from traumatic leaks to spontaneous leaks due to higher traffic safety resulting from safer vehicles, mandatory seat belts and motorcycle helmets (3,10,23,30,31,49,51).

Leak durations varied drastically from study to study, this one being no different with an average liquorrhea duration of 137.4 days, however, showing a massive span from 0 days

(where CSF leaks were caused iatrogenically and treated within the same hospital stay) up to 2920 days as a result of years of intermittent rhinoliquorrhea. Other studies showed similarly long ranges of CSF duration. Hegazy et al. (22) reported a liquorrhea-duration until diagnosis from 4 days up to 15 years, Dunn et al. (26) a duration from 4 weeks up to 6 years. However, even though the time span from the first symptoms to diagnosis had been long in some cases, the time from diagnosis to treatment (i.e. operation) in this study was on average 6.4 days, which is in line with the "International Consensus Statement: Spontaneous Cerebrospinal Fluid Rhinorrhea" by Georgalas et al.(2) who stated *"[…] that CSF leaks, even if intermittent, must be closed as soon as feasible […]"*(2).

Perioperative antibiotic treatment in this study was administered in 82.1% of cases ranging from 5 days up to 20 days. This course of action is in line with previous studies as well as the "International Consensus Statement: Spontaneous Cerebrospinal Fluid Rhinorrhea" (2,22,31).

In this study HRCT scans were able to detect CSF leaks in 67.9% of cases. While Dunn et al. (26) claimed similar results with 66%, they also included MRIs. Larger studies, including systematic reviews, reported findings regarding CT scans with sensitivities of 90% or higher (10,37).

39.3% of patients in this study received a preoperative MRI, mainly if a meningoencephalocele was suspected after initial HRCT scans. Studies suggest an even better sensitivity of MRC, a noninvasive detection method of CSF leaks (10,31,37). The potential uses of combining these imaging methods and their comparison with IF has yet to be explored further. However, as MRI might delay surgery due to long waiting times in Austria, it is not part of the routine diagnostic procedure. If HRCT does not show signs of CSF leaks despite the presence of rhinorrhea with a positive beta-trace test, the course of action at the Department of Otorhinolaryngology, Medical University of Graz is to go directly into surgery as soon as feasible, as recommended by Georgalas et al. (2).

In 91% of cases analyzed for this study a free mucosa flap was used to repair the leak. This coincides with available data deeming free tissue grafts the preferred material of choice though *"[…] there is little evidence that one [method] is superior to another."*(2) (22).

Regarding complications, in this study no perioperative adverse events could be observed. This result corresponds with current data regarding perioperative issues associated with CSF leak repairs which are estimated to be below 1% (22,26,31).

As for closure rates this study shows a success rate upon first attempt of 68% going up to 100% after a second intervention as 25% of patients had a recurrent CSF leak. Data regarding

initial surgical success vary drastically in current literature. De Jong et al. (30) reported similar findings with an average primary surgical closure rate of 79% going up to 93% after a second intervention. Dunn et al. (26) also stated primary closure rates of 80% rising to 86% upon second attempts. Systematic reviews such as the one conducted by Psaltis et al. (31) regarding the endoscopic repair of CSF leaks showed a higher success rate of 90.6% upon first attempt going up to 96.6% with reinterventions. In their meta-analysis Hegazy et al. (22) also found success rates of 90% going up to 97% counting reinterventions. Lumbar drains are infrequently mentioned in research. In the studies included in the meta-analysis by Hegazy et al. (22) perioperative lumbar drains were used 49% of the time, far more often than the 1.1% shown in this study.

Hospitalization times within this study's patient collective spanned from 2 days up to 53 days which follows the recommendation of the "International Consensus Statement: Spontaneous Cerebrospinal Fluid Rhinorrhea" regarding adequate hospitalization time while strongly discouraging day-case CSF leak repairs (2).

Comparing this study's follow-up period of an average of 14.2 days with present literature reveales a significant drawback of Austrian tertiary care hospitals which often loose contact with patients after successful treatment as further treatment is, as a rule, being outsourced to primary and secondary care facilities. Seth et al. (49) reported an average follow-up of 27.1 months, Hegazy et al. (22) "greater than 24 months"(22), Dunn et al.(26) of 98 months and Bernal-Sprekelsen et al. (55) of 65 months. For future research patient outreach regarding complications, recurrences and general satisfaction should be considered to properly assess current treatment options and outcomes.

Each patient included in this study had a beta-trace test done, all of which came back positive with an average concentration of 20.5 mg/l. Results above 1.69 mg/l were considered as "positive". This cutoff value is comparatively high when looking at current literature recommending 1.11 mg/l for a high sensitivity (over 90%) and specificity (up to 100%). As a result, actual CSF leaks may go unnoticed even though only 78.5% of patients actually had confirmed CSF leaks. This might further underline the necessity for a multimodal diagnostic pathway to accurately detect CSF leaks (2,36,37,40,41).

In this study sodium-fluorescein was administered intrathecally on average 15.2 hours before the operation. There are only a few studies mentioning the timespan between IF injection and surgery. Keerl et al.'s (52) time between application and surgery of 16-20 hours closely resembled this study's findings. Seth et al. (49) deemed 60-90 minutes, Le et al. (3) 30-60 minutes an adequate time for sodium-fluorescein to dilute within CSF. In one case of this

study (3.6%) the intraoperative ITF evaluation was negative (i.e. a leak combined with ITF associated fluorescence can be observed) despite a visible CSF leak. In 2010 Seth et al. (49) stated that false-negative results occur in up to 26% of cases, however, their average time between application and endoscopy did not exceed two hours. Whether or not longer time periods between application and endoscopy for ITF to properly distribute within CSF correlate with lower false-negative results has not been assessed yet. Overall in 75% of cases in this study the ITF evaluation was positive which closely coincides with Seth et al.'s (49) sensitivity estimation of 73.8%. Jolly et al. (53) reported a sensitivity of 99.7%.

In this study no adverse reactions associated with ITF (on average 0.7 ml were administered) were documented which matches past studies which found that side effects were rare and largely associated with higher doses of ITF (31,51–53). CSF leaks were able to be observed in 60.7% of cases before applying UV light (i.e. the ITF evaluation was not necessary for leak site detection). In 23.9% of patients with positive ITF evaluation no clear signs of CSF flow could be observed. Considering this, ITF deemed itself useful in merely 57.8% of cases. Nevertheless, ITF remains a valid option for confirming and localizing CSF leaks which is why its utilization was deemed useful for localizing leaks and ensuring successful closure (2,37). However, higher-level studies are necessary to better define the safest and most effective applications of this procedure. Some studies have shown favorable results of topical fluorescein application, though more data to support this claim has yet to be collected, especially to compare the use of ITF with topical fluorescein (5).

4.1 Sternberg's canal defect as a source of CSF leaks

The role of Sternberg's canal (or lateral pharyngeal canal), respectively its defect, as a source of spontaneous CSF leaks has been a highly discussed topic over the last few years. Originally described in 1888 by the Austrian doctor Maximilian Sternberg, this structure originates *"from the medial angle of the superior orbital fissure at the base of the outer root of the lesser wing of the sphenoid"*(33) and *"exits medial to the processus vaginalis in the nasopharynx [in its complete form] or ends in the sphenoid sinus in its incomplete form"*(33). When taking the embryogenesis of the sphenoid bone into account this represents the fusion plane of the alisphenoid, basisphenoid, and presphenoid. It is important to bear in mind that by this definition this structure lies medial to the foramen rotundum, vidian canal, and maxillary nerve. Sternberg assumed a prevalence of 4% in adult skulls while declaring the canal to be omnipresent in the skulls of children between 3 and 4 years of age (29,32,57).

A systematic review conducted by Barañano et al. (29) evaluating the presence of a lateral pharyngeal canal in-vivo was not able to support Sternberg's hypothesis. By analyzing high-resolution CT scans of 1000 healthy individuals, they merely found one defect that met the location's original definition, while also being much smaller than previous descriptions suggested (29).

About 30-50% of spontaneous CSF fistulas are considered to originate from the sphenoid sinus, possibly hinting towards some sort of predisposed structural weakness of the bone. Illing et al. (25) examined 59 cases of patients suffering from spontaneous CSF leakage, however all defects within the sphenoid sinus were located laterally of the maxillary nerve, therefore not meeting the criteria for Sternberg's canal defect. Moreover, the vast majority of patients showed radiological signs of ICP, such as empty sella and arachnoid pits while also falling into the demographic of IIH, i.e. obese, middle-aged females (3,25).

Taking all of the above into consideration, even if some sort of congenital weakness is present within the structure of the sphenoid sinus, manifest spontaneous CSF leakage is much more likely to arise in conjunction with other pathologies such as intracranial hypertension (25,32,58).

4.2 Limitations

This thesis is subject to several limitations primarily due to its retrospective nature. It relies heavily on the quality of past documentation which usually becomes less thorough going further back in time, with some data missing altogether due to the lack of digital storage for physical records. Another challenge derives from the intermittent symptoms of CSF leaks, which can make it difficult to accurately reconstruct the initial manifestations, as they may go unnoticed over extended periods of time.

Additionally, the overall short follow-up period for newer cases presents a limitation. Since the Department of Otolaryngology of the Medical University of Graz is part of a tertiary care center, patients often come from a large catchment area and may not return for follow-up if no further complications arise, resulting in a lack of long-term data. Furthermore, information regarding follow-up care is also often limited, as patients are frequently referred to non-clinical doctors or receive follow-up care outside the hospital, a common practice in Austria. Finally, the small sample size, due to the overall rarity of the condition, limits the ability to adequately estimate the sensitivity and specificity of the diagnostic tools assessed in this study. These factors collectively highlight the inherent drawbacks of retrospective

studies, where data limitations and follow-up challenges can impact the overall robustness of findings.

5 Conclusion

Both, testing for beta-trace protein in nasal discharge as well as ITF evaluation have played an important role in CSF leak detection for many years. This study has shown similar results to other published material considering the sensitivity of both diagnostic measures on their own. However, evaluating the combined use of testing for beta-trace and the ITF evaluation has shown that neither one can be substituted by the other. Testing for beta-trace is only able to detect the presence of liquorrhea and ITF is necessary for intraoperative leak localization (if no defects are visible in HRCT) as well as confirming tight closures and evaluate possible false-positive beta-trace results. In this study ITF provided a diagnostic advantage in 57.7% of cases which is modest when considering the invasive nature and possible side effects of this procedure, especially since other non-invasive measures such as topical sodium-fluorescein or MRC are available. The latter has shown very promising results leaving the question if MRC could play a more dominant role in CSF leak detection in the future, possibly eliminating the necessity for intrathecal fluorescent media and unnecessary surgeries altogether. Therefore, future studies should explore and compare various diagnostic approaches, such as combining beta-trace with HRCT and ITF or beta-trace with MRC and ITF, to determine the most accurate and minimally invasive techniques for confirming and localizing CSF leaks.

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