On the diagnosis of external ventricular drain related infections - Incidence, biomarkers and microbiological methods

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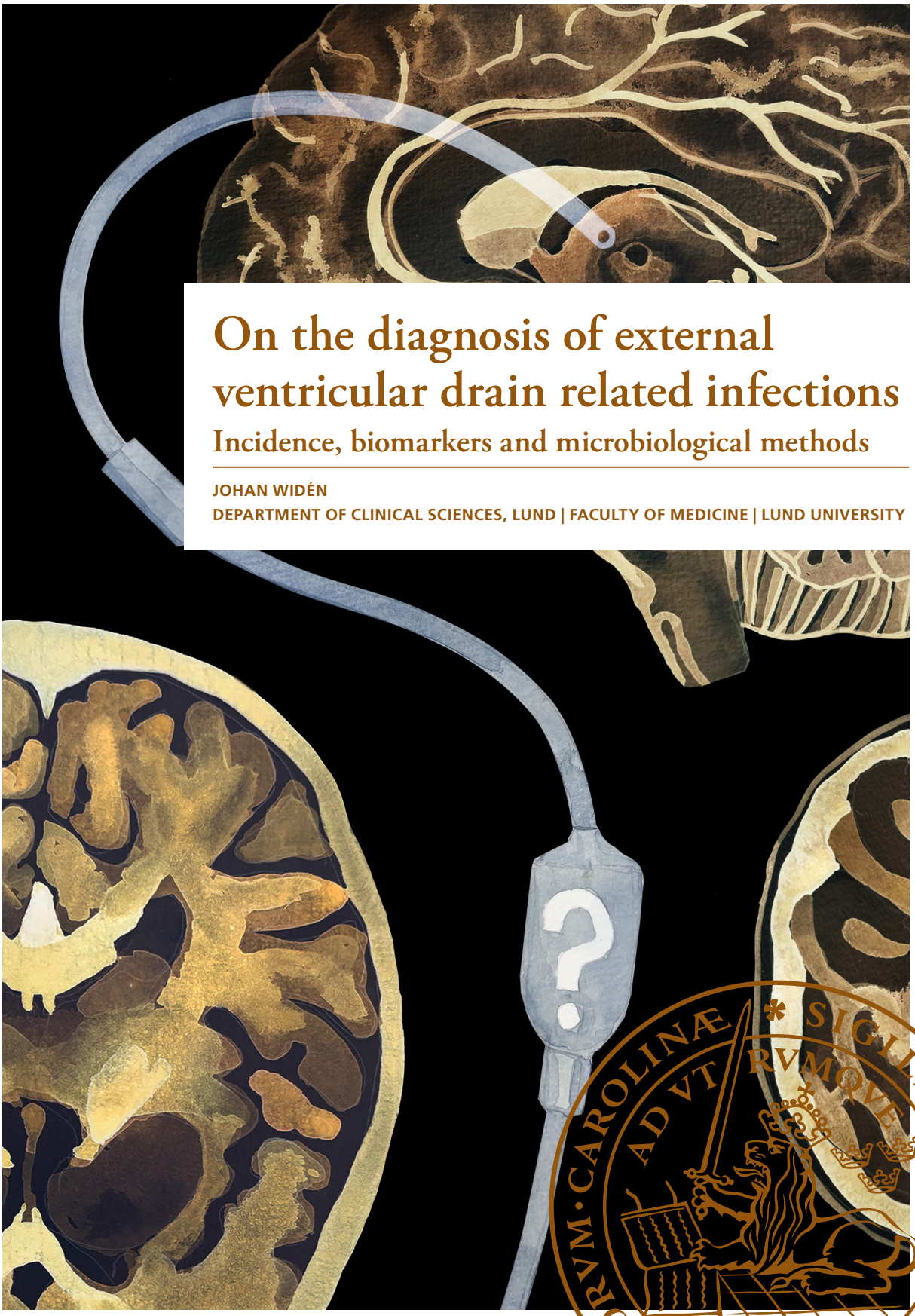

On the diagnosis of external ventricular drain related infections

Incidence, biomarkers and microbiological methods

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–Incidence, biomarkers and microbiological methods

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Incidence, biomarkers and microbiological methods

Johan Widén

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract:

The external ventricular drain (EVD) is an important instrument in the management of neuro-intensive care patients through its ability to meassure intracranial pressure and to treat increased intracranial pressure by drainage of cerebrospinal fluid (CSF). However, EVDs are associated with the risk of external ventricular drain related infection (EVDRI). EVDRI diagnosis is challenging as symtoms and CSF biomarker alterations associated with infection are unspecific in neuro-intensive care patients and reported incidence rates of EVDRI varies widely.

We investigated the incidence and microbiological aetiology of EVDRI of patients with subarachnoid haemorrhage in a Swedish setting and estimated the incidence to 5.8% or 4.1 per 1000 EVD days. However, over 40% of subjects received treatment for EVDRI. The majority of EVDRI's were caused by coagulase-negative staphylococci (Paper I). The diagnostic performance of the neutrophil protein heparin-binding protein (HBP) was assesed as a CSF biomarker for EVDRI in a separate cohort of neuro-intensive care patients. HBP was found to be significantly higher in EVDRI but with considerable overlap to non-EVDRI subjects, resulting in a limited sensitivity of 86% and a specificity 76% for EVDRI (Paper II). Furthermore, the performance of polymerase chain reaction (PCR) of the 16S rRNA gene for the diagnosis of EVDRI was evaluated. This method showed a good concordance to bacterial culture of CSF and the results could be used to direct decisions regarding antimicrobial therapy prior to culture results. Additionally. the results did not indicate that false negative CSF cultures are common in patients treated with an EVD (Paper III). Finally we attempted to characterise the CSF proteome of EVDRI with mass spectrometry. However, no individual proteins were differentially expressed between subjects with and without EVDRI, probably due to large heterogeity of the CSF proteome of neuro-ICU patients, related to their underlying condition.

In summary, this thesis explores different aspects of EVDRI diagnosis and highlights the need for improved diagnostic methods. The results imply that finding sensitive and specific biomarkers related to the host response will be challenging due to the heterogeneity of the CSF proteome, whereas improved microbiological methods could improve EVDRI diagnostics.

Key words: External ventricular drain, Ventriculostomy related infection. Neurocritical care. 16S PCR, Third generation sequencing. CSF proteomics

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We live on an island surrounded by a sea of ignorance. As our island of knowledge grows, so does the shore of our ignorance.

- John Archibald Wheeler

Yet here poor fool for all my lore, I stand no wiser than before

-Johann Wolfgang von Goethe

Table of Contents

Populärvetenskaplig sammanfattning

Varje år vårdas cirka 1500–2000 patienter på Sveriges neurokirurgiska intensivvårdsavdelningar (NIVA) i samband med akut sjukdom i centrala nervsystemet (CNS) eller efter planerade neurokirurgiska operationer. Akuta orsaker till inläggning på NIVA är till exempel olika typer av hjärnblödningar och hjärnskada till följd av exempelvis trafikolyckor. Omhändertagandet av patienter på NIVA handlar till stor del om att se till att hjärnan har optimala förutsättningar att läka och att ingen ytterligare skada uppstår i efterförloppet. För att säkerställa detta krävs kontinuerlig övervakning av patienternas andning och cirkulation, men också specifik övervakning av förhållandena för hjärnan, såsom intrakraniellt tryck (ICP). Komplikationer som kan uppstå är bland annat att patienten drabbas av en ny blödning (re-blödning), sammandragning av hjärnans blodkärl (så kallad vasospasm), svullnad av de skadade delarna av hjärnan, krampanfall eller infektion. Dessa komplikationer kan öka trycket i hjärnan, minska blodtillförseln och/eller öka syrgasförbrukning och ämnesomsättning i hjärnan. Detta leder till försämrad syrgastillgång för hjärnans celler vilket kan leda till ytterligare hjärnskada och försämrad läkning. Dessa händelser kallas med en gemensam term för sekundära insulter.

Ett viktigt instrument vid vård på NIVA är externt ventrikeldränage (EVD). Detta är en kateter som genom en operation läggs in i en av hjärnans sidoventriklar, vätskefyllda hålrum centralt i hjärnan, och mäter trycket där. Katetern kan också användas för att låta vätskan i ventriklarna, cerebrospinalvätska (CSV), rinna ut genom katetern till ett uppsamlingssystem utanför patienten. Detta är användbart om den normala cirkulationen av CSV från sidoventriklarna är blockerad till följd av till exempel blödning eller svullnad. Genom att låta CSV dräneras ut ur skallen kan EVD-katetern användas för att sänka trycket i hjärnan. Vårdpersonalen kan ställa in vid vilket tryck som vätska ska börja dräneras för att sänka trycket.

En nackdel med EVD är att flera naturliga barriärer för infektion bryts och att katetern kan fungera som inkörsport för bakterieinfektioner på hjärnans eller ventriklarnas yta, så kallad meningit eller ventrikulit. Dessa infektioner kallas EVD-relaterade infektioner (EVDRI). Infektioner i hjärnan är ofta allvarliga tillstånd som kliniskt verksamma läkare har stor respekt för. Vid akut bakteriell meningit (ABM), som uppkommer utan EVD eller NIVA-vård, är dödligheten 10–20% trots modern sjukvård. Risken för bestående hjärnskador med funktionsnedsättning är också stor. På grund av detta och mot bakgrunden att man vid NIVA-vård vill undvika sekundära insulter så är det uppenbart att man så långt det går vill undvika dessa infektioner och behandla dem i ett tidigt skede om de uppstår.

Ett problem är att de symtom som kan tyda på bakteriell meningit, såsom feber, påverkad medvetandegrad och nackstelhet ofta förekommer även hos NIVA-patienter

utan infektion, till följd av det underliggande tillståndet och/eller att patienten hålls sövd. Det vanligaste sättet att påvisa bakteriell meningit är att ta prov på CSV. Vid infektion kan man se ökat antal vita blodkroppar (leukocyter) samt förhöjda halter av laktat och protein och sänkt nivå av glukos i CSV. Tyvärr förekommer dessa tecken ofta hos patienter på NIVA, även utan infektion, vilket ytterligare försvårar diagnostiken av EVDRI. För att definitivt bekräfta en infektion så försöker man odla fram bakterier från CSV på ett mikrobiologiskt laboratorium. Detta tar i normalfallet flera dygn och därför måste man ofta fatta beslut om att sätta in behandling för misstänkt meningit baserat på mängden vita blodkroppar, laktat och protein, före svaren på odlingar är tillgängliga. Dessvärre är inte heller resultaten från odling helt tillförlitliga, då de bakterier som odlas fram vid misstanke om EVDRI ofta är mindre sjukdomsalstrande hudbakterier som också kan vara föroreningar vid provtagningen eller kan växa på plastmaterial utan att orsaka infektion. Det faktum att många av patienterna på NIVA behandlas med antibiotika för andra infektioner gör också att det finns en risk att det är svårt att odla fram bakterier från CSV även vid en faktisk infektion.

Under de senaste decennierna har så kallad polymerase chain reaction-teknik (PCR), där arvsmassa, deoxiribonukleinsyra (DNA) eller ribonukleinsyra (RNA), med en speciell sekvens kan påvisas, kommit att bli allt viktigare inom mikrobiologisk diagnostik. PCR-test för en gen som är unik för den bakteriella cellen, så kallad 16S PCR, kan påvisa en stor mängd olika bakteriearter och analysen tar oftast kortare tid än bakterieodling. Genom att kartlägga den exakta sekvensen av DNA i 16S-arvsmassan vid ett positivt PCR-resultat kan man också ofta fastställa vilken bakterie som finns i ett prov. Denna teknik används på CSV vid diagnostik av EVDRI men det är dåligt studerat hur väl den fungerar i denna situation. Sammantaget leder dessa begränsningar i diagnostiken av EVDRI till att man ofta väljer att behandla en infektion utan att det är säkert att det faktiskt finns en, för att inte riskera ett försämrat utfall för patienten.

Behandlingen av EVDRI består av bredspektrumantibiotika, oftast två olika preparat i kombination. Behandlingen kan dels leda till biverkningar och förlängd vårdtid för den enskilda patienten men användningen riskerar också att driva fram utveckling av antibiotikaresistenta bakterier, både lokalt på NIVA och i samhället i stort. Det är därför viktigt att förbättra diagnostiken av EVDRI för att säkerställa snabb och korrekt behandling till svårt sjuka patienter samt undvika onödig användning av bredspektrumantibiotika till patienter utan infektion.

Denna avhandling innehåller fyra delarbeten som alla i förlängningen syftar till ökad kunskap om diagnostik av EVDRI. I det första arbetet (I) gjordes en undersökning av hur vanligt förekommande EVDRI är hos patienter med subaraknoidalblödning (SAH), en allvarlig typ av hjärnblödning, som vårdats på NIVA och behandlats med EVD. Arbetet visade att säkerställd EVDRI var relativt sällsynt (5,8% av patienterna) men att man ofta misstänkte EVDRI och att många av patienterna (42,4%) någon gång

behandlades för EVDRI under tiden på NIVA. Det visades också att det ofta gjordes avsteg från lokala riktlinjer avseende när EVDRI skulle misstänkas eller behandlas och att dessa riktlinjer inte var tillräckligt träffsäkra. Det sågs också att så kallade koagulasnegativa stafylokocker (KNS), som normalt hittas på huden, var de vanligaste bakterierna som påvisades vid EVDRI.

Det andra arbetet (II) undersökte om proteinet heparinbindande protein (HBP) kan användas som biomarkör för EVDRI. Resultaten visade att HBP-koncentrationen i CSV var signifikant högre hos patienter med EVDRI och att känsligheten för att identifiera en patient med EVDRI var 86% och specificiteten 76%. Säkerheten i resultaten begränsades av att endast 7 av de 90 analyserade patienterna utvecklade EVDRI. Man såg också att HBP varierade mellan olika patientgrupper på NIVA och var högst hos de patienter som lades in på grund av ABM.

I delarbete tre (III) undersöktes hur väl bakteriepåvisning med PCR överensstämde med odlingsresultat i CSV hos patienter EVD. Sammantaget sågs att PCR stämde med odlingsresultat i 77% av patienter med positiv odling och i 98% av de med negativ odling. Möjligen kan vissa av de fall av positiv odling där 16S PCR var negativ utgöras av falskt positiva odlingsresultat pga. kontamination vid provtagning eller på laboratoriet. Vi såg också att bakterieidentifiering med ny sekvenseringsteknik, Nanopore-sekvensering, överensstämde med artbestämning av de framodlade bakterierna i 91% av fallen. Genom att analysera mängden 16S DNA i alla prover kunde vi också konstatera att det inte finns några tydliga tecken på att bakterieodlingarna är falskt negativa hos patienter med pågående antibiotikabehandling vid provtagning, då mängden 16S DNA i odlingsnegativa prover inte skiljde sig från nivåerna som uppmättes i negativa kontrollprover.

Det fjärde arbetet (IV) utfördes på samma grupp patienter som det tredje men här var även en liten grupp med patienter med ABM inkluderad. Patienterna med ABM hade lagts in på NIVA för att få behandling med EVD, vilket görs i de allvarligaste fallen. I studien analyserades insamlad CSV med så kallad masspektrometri (MS) som snabbt kan mäta mängden av tusentals olika proteiner i ett prov, det så kallade proteomet. Proteomet i CSV hos patienter med EVDRI respektive ABM jämfördes sedan med CSV-proteomet hos de personer i studiegruppen som inte visade några tecken på CNS-infektion. Efter statistisk analys sågs inga enskilda proteiner där mängden var signifikant högre eller lägre i CSV hos patienter med EVDRI. Hos patienter med ABM sågs större skillnader i proteomet men inte heller här var skillnaden i mängd av något enskilt protein statistiskt signifikant jämfört med de patienter som inte hade någon infektion.

List of Papers

Paper I

Widén J, Eriksson B-M, Ronne-Engström E, Enblad P, Westman G. Ventriculostomy-related infections in subarachnoid hemorrhage patients - A retrospective study of incidence, etiology, and antimicrobial therapy. Acta Neurochir (Wien) 2017;159:317–23. doi: 10.1007/s00701-016-3039-2.

Paper II

Widén J, Cederberg D, Linder A, Westman G. Heparin-binding protein as a marker of ventriculostomy related infection and central nervous system inflammation in neuro-intensive care. Clin Neurol Neurosurg 2023;229:107752. doi:10.1016/j.clineuro.2023.107752

Paper III

Widén J, Morén J, Mölling P, Fagerström A, Enblad P, Eriksson BM, Ronne-Engström E, Sundqvist M, Westman G. Diagnosis of external ventricular drainage related infections with real-time 16S PCR and third-generation 16S sequencing. Infect Dis (Lond). 2024 Mar 26:1-10. doi: 10.1080/23744235.2024.2331260

Paper IV

Widén J, Morén J, Enblad P, Eriksson B-M, Ronne-Engström E, Linder A, Bakoshi A, Årman F, Westman G. Proteomic characterization of CSF in EVDRI during neuro-intensive care. Manuscript unpublished.

Abbreviations

ABM	Acute bacterial meningitis
AI	Antibiotic impregnated
ASC/AST	Active surveillance culture/testing
AUC	Area under curve
CBF	Cerebral blood flow
CDC	Centre for Disease Control
CI	Cell-index
CI	Confidence interval
CoNS	Coagulase-negative staphylococci
Cp	Crossing point
CPP	Cerebral perfusion pressure
CSF	Cerebrospinal fluid
DDA	Data-dependant acquisition
DIA	Data-independent acquisition
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ESI	Electro-spray ionization
EVD	External ventricular drain
EVDRI	External ventricular drain-related infection
FDR	False discovery rate
GCS	Glasgow coma scale
GOS	Glasgow outcome scale
GOSE	Glasgow outcome scale extended
HBP	Heparin-binding protein
HCAMV	Healthcare associated meningitis and ventriculitis
ICP	Intracranial pressure
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
IgM/G	Immunoglobulin M/G
IL-6	Interleukin-6
IMCU	Intermediary care unit
IQR	Interquartile range
IVH	Intraventricular haemorrhage

LC	Liquid chromatography
LC-MS/MS	Liquid chromatography tandem mass spectrometry
m/z	Mass to charge-ratio
MAP	Mean arterial pressure
mRS	Modified Rankin scale
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
NaCl	Sodium chloride
NGS	Next-generation sequencing
NHSN	National Healthcare Safety Network
NIC	Neuro-intensive care
NICU	Neuro-intensive care unit
OR	Odds ratio
PASEF	Parallel accumulation of serial fragmentation
PCR	Polymerase chain reaction
PMNC	Polymorphonuclear cells
PNBM	Post-neurosurgery bacterial meningitis
RBC	Red blood cell count
RCT	Randomised controlled trial
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
rRNA	Ribosomal ribonucleic acid
SAH	Subarachnoid haemorrhage
SC	Silver coated
SI	Shunt infection
SILF	Svenska infektionsläkarföreningen
TBI	Traumatic brain injury
VAI	Ventriculostomy-associated infection
VRI	Ventriculostomy-related infection
WBC	White blood cell count

Introduction

Neuro-intensive care (NIC) is focused on optimising the conditions for the brain to recover from the condition or injury leading to neuro-intensive care unit (neuro-ICU or NICU) admission (the primary insult). This is achieved by extensive monitoring and managing of cerebral blood flow and oxygenation while preventing and treating cerebral complications (secondary insults) at an early stage. Secondary insults are diverse processes that all lead to either increased intracranial pressure and/or reduced cerebral blood flow and/or increased brain metabolism in different combinations. Ultimately, these processes lead to an imbalance between the demand and availability of oxygen in the brain, resulting in increased damage to the brain and a worse outcome for the patient.

One of several key instruments in neuro-intensive care is the external ventricular drain (EVD) also known as a ventriculostomy catheter. Placement of this device is often one of the first measures in the management of a patient with an acute neurosurgical condition that is admitted to the neuro-ICU. The EVD allows for both measurement of intracranial pressure (ICP) as well as therapeutic lowering of ICP by drainage of cerebrospinal fluid (CSF). However, the EVD also breaks natural barriers against infection and can act as a surface for bacterial growth. Hence, EVDs are associated with the risk of healthcare-associated infection, more specifically infection of the meninges and/or the ventricles, meningitis or ventriculitis. When caused by an EVD these infections are called EVD related infections (EVDRI) or ventriculostomy related infections (VRI).

When acquired outside of healthcare, acute bacterial meningitis (ABM) is a devastating condition with mortality rates of 10–20% despite modern healthcare. Hence, healthcare associated meningitis, such as an EVDRI represents a potent secondary insult and should be diagnosed and treated promptly. This has proven a challenging task since the symptoms and CSF biomarker alterations associated with CNS infections can also be caused by the underlying condition. This can lead to overuse of broad-spectrum antibiotics, delayed diagnosis and treatment, prolonged duration of NIC and increased healthcare costs and suffering. To further improve the management of NIC patients while limiting unnecessary and potentially harmful antibiotic treatment, increased knowledge regarding the incidence and diagnostics of EVDRI is important.

Background

Neuro-intensive care

History

During the 1970s and 80s the knowledge of the processes occurring after traumatic brain injury (TBI) increased rapidly. It was shown that elevated intracranial pressure (ICP) leading to a decreased cerebral perfusion pressure (CPP) and a resulting reduction in cerebral blood flow (CBF) and oxygen delivery were major factors contributing to death or an unfavourable outcome after head injury and that ICP should be monitored and lowered if elevated[1,2]. Additionally, autopsy studies of patients with traumatic head injury identified that swelling of the brain parenchyma, signs of hypoxic brain damage, epilepsy or meningitis were related to mortality in patients with a "talk and die" clinical course where the patient was initially well but later deteriorated and died[3,4]. Additional research identified and emphasised that several factors such as hypotension, hypoxemia, seizures, fever, and infections were associated with a negative outcome in patients with head injury and established the concepts of secondary insults and avoidable factors that should be monitored for and avoided to enhance the prognosis of patients with TBI[5–7]. Based on these insights, the concept of specialized neurointensive care, performed in a specific neurointensive care unit (neuro-ICU), was established. In Swedish healthcare these concepts were implemented during the 1980s and 1990s and have been shown to improve the outcome of patients with head injuries[8,9].

Subarachnoid haemorrhage

In addition to TBI, subarachnoid haemorrhage (SAH) is one of the major causes of NIC admission. SAH is categorized as a form of stroke and constitutes approximately 5% of stroke cases but more often affects younger persons and has higher mortality rates than other forms of stroke[10]. The vast majority of SAH cases (85%) are due to rupture of an aneurysm on the cerebral arteries adjacent to the circle of Willis. This causes a sudden onset of symptoms such as severe headache, vomiting, focal neurological deficits, and in more severe cases, loss of consciousness[11].

An important aspect of SAH management is prevention of ICP elevation due to post haemorrhagic hydrocephalus, caused by blood blocking the circulation of cerebrospinal fluid (CSF). This has been shown to occur in 15–58.4% of SAH patients[12] and can be treated by placement of an external ventricular drain (EVD) in one of the lateral ventricles of the brain[13]. The implications of EVD treatment will be discussed in detail in following chapters.

The external ventricular drain

History

The EVD that is used in NIC today refers to a device that serves two purposes, measuring ICP and allowing CSF to be drained out of the lateral ventricles. The concept of draining CSF to treat congenital hydrocephalus by an external drain was first documented in 1744 by Claude-Nicolas Le Cat. During the following 150 years it was used infrequently, mainly to treat congenital hydrocephalus in paediatric patients, and its use would not be expanded until the importance of ICP was recognized during the second half of the 20th century[14].

The consequences of increased ICP were described by Harvey Cushing during the first years of the 20th century[15]. Although ICP measurement by lumbar puncture was performed earlier, measurement of ICP in the ventricles was first described by Hodgson in 1928[16]. Continuous measurement of ICP was first described in the 1950s[17,18]. Simultaneously, scientific reports of EVD-devices used to drain CSF from the lateral ventricles in patients with increased ICP were published[19,20]. In the 1960s, Nils Lundberg at the Department of Neurological Surgery in Lund further developed the technique for continuous ICP-monitoring and made important contributions to the knowledge of ICP physiology[21,22]. Lundberg's method of ICP monitoring also allowed for simultaneous CSF drainage and the device he used was essentially what we now refer to as an EVD. During the following decades the body of evidence for ICP monitoring and management has grown exponentially, and ICP-monitoring is now standard practice and recommended in the international management guidelines for TBI[23]. In SAH, CSF diversion and ICP monitoring by an EVD is recommended in patients with post haemorrhagic hydrocephalus[24]. Technical advances in microtransducers have allowed for smaller and more accurate pressure monitors that can be placed in the brain parenchyma, however, if CSF drainage is or might become necessary, an EVD is still the first choice for ICP monitoring[18].

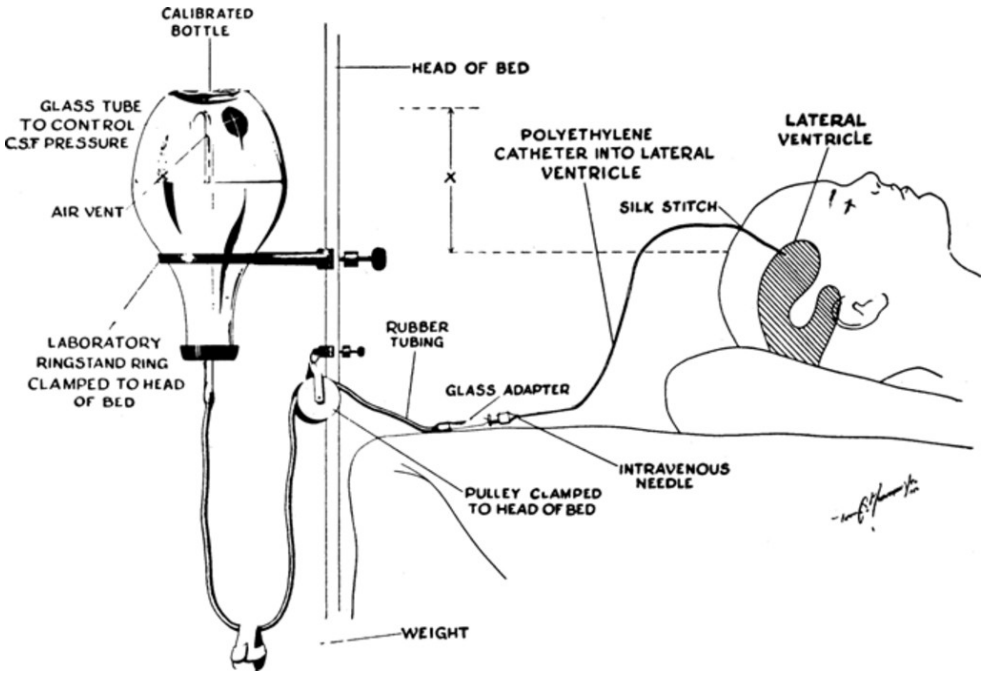

Figure 1: An early illustration of an EVD device published in 1951.
Originally published in Bering, E. A., Jr. (1951). A Simplified Apparatus for Constant Ventricular Drainage. Journal of Neurosurgery, 8(4), 450-452. Reprinted with permission.

Surgical procedure

Placement of an EVD is one of the most common neurosurgical procedures[25,26]. It is commonly performed in the operating room by a neurosurgeon but could also be performed bedside in the neuro-ICU by either an intensivist or neurosurgeon and there is variation between different centres regarding the routines for the procedure[27]. The surgical procedure of placing a drain in the lateral ventricle was developed during the 19th century by William Williams Keen and refined by the German surgeon Hermann Tillmanns, who advocated the use of Kocher's point as the site of EVD placement[14]. The procedure was further refined by Friedman and Vries who introduced tunnelling the ventricular catheter through the scalp several cm before exiting the skin, to decrease the risk of infection[28].

Kocher's point is still the most used point of entry for an EVD. This point is located approximately 3 cm lateral to the midline and 10-11 cm posterior from the glabella or 1-2 cm anterior to the coronal suture. A skin incision is made and after reaching bone, the periost is scraped before a twist-drill is used to make a hole through the cranium. The meninges are then opened sharply before the EVD-catheter is placed through the brain tissue, aimed at the frontal horn of the ipsilateral ventricle. The direction of the

catheter is usually determined by freehand pass technique using anatomical landmarks. After correct placement is confirmed when CSF is returned through the catheter, it is tunnelled 4-6 cm before exiting the skin. It is then secured by sutures before being draped according to local routine. The catheter is then connected to an external draining system with a pressure monitoring device and ready for use[29,30].

Figure 2: Photographs of EVD details.
Top left: Patient (actor) with EVD connected to drainage and pressure monitoring device bedside. Top right: Details of EVD setup bedside. A: CSF drainage system and collection bag. B: Pressure monitoring device levelled at the height of the patients ventricles aided by a laser indicator. Bottom: EVD catheter after insertion at the operating room, before (left) and after (right) draping. Photographs by Gunnar Gunnarsson, Section of Neurosurgery, Department of Clinical Sciences, Lund University.

Infections related to EVDs

Introduction and incidence

The risk of iatrogenic CNS infection has been a concern with EVD-treatment since it was first introduced. Based on the high mortality and morbidity observed in ABM[31], it was evident that bacterial infection of the meninges or ventricles should be avoided at all costs in patients already suffering from neurologic disease or injury. With the introduction of the concept of secondary insults, including fever and infections, this was further emphasized[5].

The terminology for infections related to EVDs has not been consistent. The terms ventriculostomy-related/associated infections (VRI/VAI) have been frequently used, the term ventriculostomy, however, is seldom used to refer to an EVD in clinical practice and could in principle refer to both an EVD and a permanent CSF shunt. Hence, EVDRI is a more specific term. Both VRI and EVDRI could theoretically refer to both superficial soft tissue infections and deeper infections of the meninges or ventricles related to the EVD but has primarily referred to the latter. Other terms, such as health care associated meningitis and ventriculitis (HCAMV) or simply ventriculitis are also in use. Additionally, EVDRI is also sometimes included in the term post-neurosurgical bacterial meningitis (PNBM) which also includes cases of meningitis after neurosurgery in the absence of EVD-treatment. The term EVDRI will be used in this thesis summary and will refer to bacterial infection of the meninges or ventricles in the presence of an EVD. In paper I and II, however, the term VRI is used and is interchangeable with EVDRI.

Lundberg and colleagues performed daily bacterial cultures and cell counts of CSF in the patients where ICP was measured. They stated that they observed no sign of infection and concluded that "our technique permits recording of ventricular fluid pressure for long periods practically without risk of infection"[21]. However, other neurosurgeons had different experiences regarding infections and proposed alternative means for ICP-measurement, such as the subarachnoid screw, to reduce the risk of infection[32]. The technique of subcutaneous tunnelling of the EVD catheter was specifically developed to reduce the incidence of infections[28]. The use of prophylactic antibiotics to reduce infection frequency was also advocated at an early stage, with a study showing a lower incidence of 9% in patients with prophylactic antibiotics compared to 27% in the control group[33].

Rates of infection varied significantly between studies and the first large review of the subject in 2002 concluded that infection rates ranged between 0–22% per patient, although differences in definition of infection decreased comparability. When pooling data from 5261 patients in 23 studies the rate of positive CSF cultures was 8.8% per

patient and 8.08% per EVD[34]. A later meta-analysis found 752 EVDRIs in 6681 patients (11.2%) and calculated the incidence of EVDRI per 1000 EVD days to 11.4[35].

Table 1: Examples of reported rates of EVDRI in studies from different settings and times.

Study (author, year)	Country	Incidence (% of patients)	Incidence (per 1000 EVD days)
Alleyne et al. (2000)[36]	United States	3.9	4.2
Camacho et al. (2011)[37]	Brazil	18.5	21.5
Hoefnagel et al. (2008)[38]	Germany	23.2	28.7
Holloway et al. (1996)[39]	United States	10.5	13.9
Kim et al. (2012)[40]	South Korea	3.5	2.8
Schultz et al. (1993)[41]	United States	20.5	17.2
Williams et al. (2011)[42]	Australia	14.1	26.4
Wyler and Kelly (1972)[33]	United States	15.7	31.4

Risk factors

Duration of EVD treatment

EVD treatment duration is an obvious risk factor for EVDRI, as longer EVD treatment means longer risk exposure for EVDRI. Whether the risk of EVDRI per day is constant, increases or decreases with the length of treatment has been debated, with conflicting result in different studies. Several studies have shown an increasing rate of infection during EVDRI treatment[38,39,41,43–46], whereas others have found constant or declining rates over time[47–51]. The meta-analysis by Ramanan et al. showed a lower incidence per catheter day in studies with longer duration of treatment[35] suggesting a higher risk in the initial period of EVD treatment. The reason for this discrepancy in findings may be that EVDRI are caused both by bacterial contamination during EVD insertion and by bacterial colonisation and invasion by the EVD catheter at a later stage, and that the relative contribution of these two mechanisms vary, depending on local routines for EVD insertion and management.

Cause of admission

SAH and intraventricular haemorrhage (IVH) was consistently identified as risk factors for EVDRI compared to other diagnoses in early studies[38,39,47,52,53]. Although later studies show some heterogeneity, a recent meta-analysis confirmed SAH and IVH as independent risk factors[54]. It can be speculated that the blood in the CSF of these patients acts at a substrate for bacterial growth. Also, the effect of EVDRI definitions requiring an elevated leukocyte count in CSF could mean findings of bacteria in CSF cultures will more likely be interpreted as infection in these patients due to blood, including leukocytes, in the CSF.

CSF leakage

The presence of a CSF leak from the EVD insertion site has consistently been shown to increase the risk of EVDRI with odds ratios (ORs) of up to 13[55–59].

EVD manipulation

Although routine EVD exchange was advocated at an early stage[45], EVD exchange has later been shown to be a risk factor of EVDRI[39,49,55,60–62]. Irrigation of the EVD has also been shown to be associated with an increased EVDRI incidence in early studies[45,52]. The practice of EVDRI irrigation seems to be less employed in recent years as it is often not reported in newer studies of EVDRI risk factors. The frequency of CSF sampling from the EVD has also been shown to correlate with infection[38,42,63]. Whether there is a causal relationship between sampling and infection is not known, as the association could also be due to more infections being diagnosed with increased sampling, or that patients with risk factors for infections are more frequently sampled.

Other neurosurgical procedures

Several studies have shown an increased risk of EVDRI if the patient has also undergone craniotomy in adjunction to the EVDRI placement[41,43,45,47,61,64]. However, a recent meta-analysis showed a modest OR of 1.76 and some heterogeneity in findings[54].

Systemic infection

Lozier et al. proposed systemic infection as a risk factor for EVDRI in their 2002 review, based on three early studies[34,39,41,65]. Later studies are not consistent, and some have not reported significant associations between systemic infection and EVDRI[40,66], while others have[64,67]. There is also heterogeneity regarding whether the EVDRI and concomitant infection were caused by the same bacteria or not.

Symptoms

The classical clinical symptoms of bacterial meningitis are headache, fever, nuchal rigidity, and reduced level of conscience. Unfortunately, these symptoms are common in NIC patients regardless of infection. Furthermore, clinical assessment regarding conscience, nuchal rigidity and headache is impaired when patients are sedated, which is most often the case in NIC. Fever has been observed in almost 50% of NIC patients and even more frequently when not including patients with spinal disorders[68]. Small studies have not been able to show differences in clinical parameters regarding fever, nuchal stiffness, level of conscience and presence of headache between patients with and without EVDRI[69,70]. A more recent longitudinal retrospective study demonstrated changes in body temperature and Glasgow coma scale (GCS) at the onset of EVDRI compared to earlier measurements in the same patients, indicating that monitoring of

these parameters is still valuable for EVDRI diagnosis[71]. A meta-analysis of studies reporting symptoms in patients with EVRDI showed fever was more common in patients with EVDRI (72% vs 29%) but decline in consciousness was not significantly different between patients with and without EVDRI (38% vs 41%)[72].

CSF Biomarkers

Introduction

There have been considerable scientific efforts to clarify how clinical CSF biomarkers are affected by EVDRI and how they can be used diagnostically. However, results are often conflicting and significant heterogeneity in study populations and infection definitions reduce comparability. The presence of intraventricular blood due to haemorrhage or surgery is a major confounding factor when interpreting results of CSF samples of NIC patients.

Leukocyte count

CSF leukocyte count is also known as white blood cell count (WBC). There are several studies showing a significantly higher WBC in patients with EVDRI[45,47,73–75], or in longitudinal studies, higher WBC at EVDRI onset than before[71,76]. However, there is generally a large overlap in WBC between EVDRI and non-EVDRI patients, making establishment of a cut-off value for EVDRI challenging. This is illustrated in Figure 3, from a prospective study where all CSF WBC results at different times of EVD treatment were plotted and then compared to samples with a positive CSF culture[73]. Also, since many definitions of EVDRI require an elevated WBC for EVDRI diagnosis, sound assessment of WBC sensitivity and specificity cannot be performed.

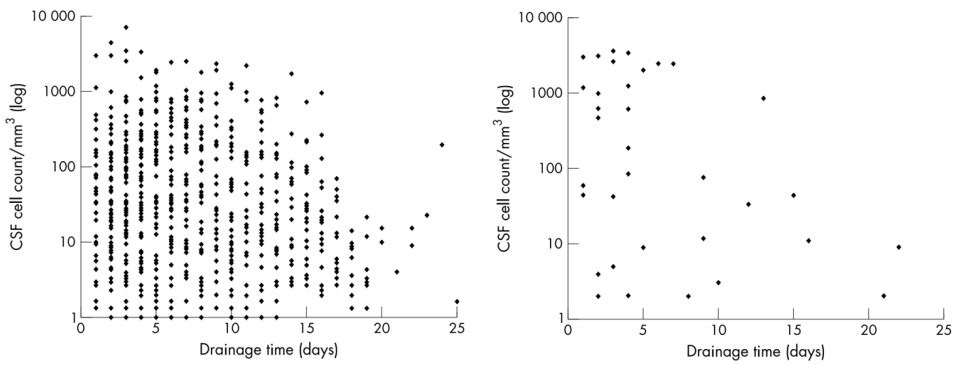

Figure 3: CSF WBC samples (left) and culture positive samples (right) at day of drainage. Figures from: Pfisterer W, Muhlbauer M, Czech T, Reinprecht A. Early diagnosis of external ventricular drainage infection: results of a prospective study. J Neurol Neurosurg Psychiatry. 2003;74(7):929. Reprinted with permission.

Based on the observations of CSF composition in community-acquired CNS infections, where polymorphonuclear leukocytes are generally more associated with bacterial infections than monomorphonuclear leukocytes[77], this has often been assumed to apply to EVDRI as well. However, there are few studies investigating whether this assumption is correct, and some existing data suggests that polymorphonuclear leukocytes is not a better predictor of EVDRI than total WBC[76,78].

A recent study by Bådholm et al. has raised further questions regarding the interpretation of CSF WBC in NIC[79]. In this study repeated CSF samples with a 15-minute interval showed considerable inter-sample variability which was further aggravated when the patient was repositioned between samplings. The authors concluded WBC variability is a major confounding factor in the diagnosis of EVDRI. The results are shown in Figure 4.

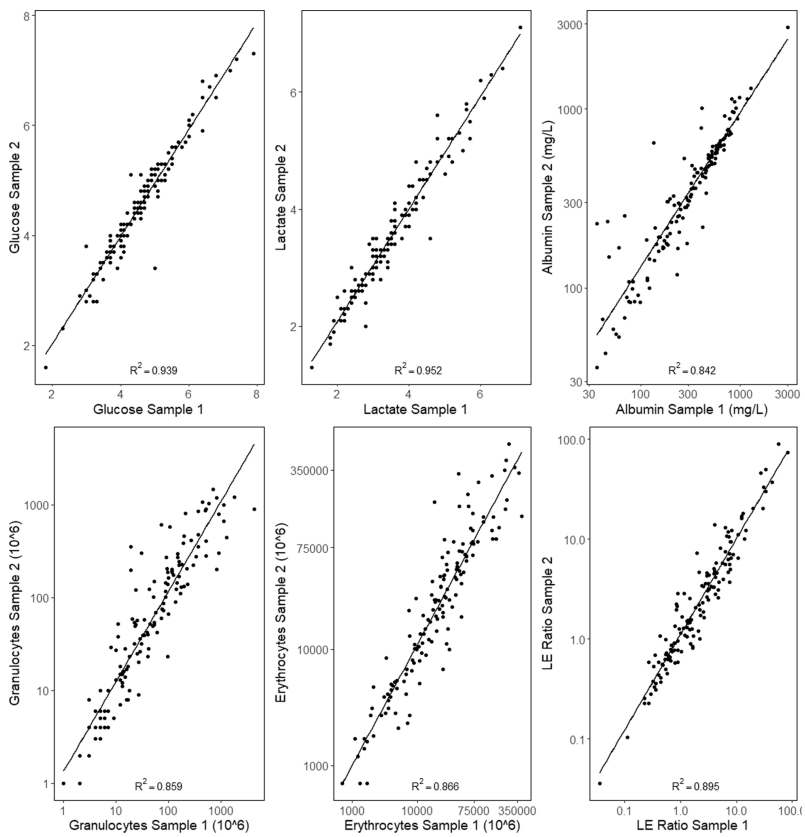

Figure 4: CSF biomarker variability in paired samples with a 15-minute interval.
Figure from: Bådholm M, Blixt J, Glimåker M, Ternhag A, Hedlund J, Nelson DW. Cerebrospinal fluid cell count variability is a major confounding factor in external ventricular drain-associated infection surveillance diagnostics: a prospective observational study. Crit Care. 2021;25(1). Original work licenced under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

Erythrocyte corrected WBC and cell-index (CI)

Since bleeding into the ventricles will carry both red and white blood cells into CSF, adjusting the CSF WBC based on the red blood cell count (RBC) has been suggested as a way of increasing the specificity of CSF WBC. The most common approach is to subtract 1 or 2 WBC for every 1000 RBC in CSF[80]. Another suggested way of increasing the specificity is by calculating a "cell-index" (CI) by dividing the ratio of WBC/RBC in CSF to the same ratio in blood where an increased CI would be indicative of EVDRI.

The CI was first suggested by Pfausler and colleagues in an observational study of 13 EVD patients where 7 developed infections, and an increased CI was observed 1–3 days before positive CSF culture[81]. Based on these findings, a cut-off of 5 for CI was later suggested as indicative of infection[82]. A subsequent study of 34 patients, where 20 were defined as EVDRI calculated a sensitivity and specificity at 95 and 92.9%, respectively for a CI cut-off of 2.9. This study used a definition for EVDRI not requiring a positive CSF culture[83]. Another study of 95 ubjects with 7 subjects defined as EVDRI by positive culture, showed a higher CI in the days preceding positive culture of EVDRI subjects than in non-EVDRI controls. This study suggested an optimal cut-off of 10.4 and did not compare the CI to other diagnostic biomarkers[84].

One case control study has demonstrated an improvement in diagnostic accuracy, reflected in an increased area under the receiver operating characteristic curve (AUC of ROC), for CSF WBC when corrected for RBC as well as for CI compared to WBC[74]. In a recent observational prospective study, the median CI was significantly higher in patients with EVDRI (21.3 vs. 0.9)[85]. Conversely, an investigation of 724 blood contaminated CSF samples from EVD patients showed a reduction in AUC of ROC for diagnosing infection when using the RBC correction method, and that there was a tendency for over-correction using this method[86]. This tendency was confirmed in a large descriptive study of temporal patterns of CSF biomarkers in 464 patients with IVH where the observed difference in CSF WBC between infected and non-infected patients disappeared when using RBC correction[87]. Furthermore, it has also been shown that after SAH, CSF WBC and RBC levels do not follow the same course over time, which would complicate WBC correction based on RBC[87,88]. Although there may be theoretical rationale for correcting CSF WBC or using the CI, the scientific support for this practice is limited.

Lactate

Lactate in CSF has been proposed as a reliable marker for EVDRI or PNBM in several studies[78,89–92]. Leib et al. first suggested a CSF lactate of >4 mmol/L to be indicative of infection[89]. The CSF samples collected in this study were obtained by lumbar puncture on patients with suspected PNBM in patients without EVD, reducing its applicability on EVDRI. Also subjects with negative CSF culture were divided into presumed- and

no bacterial meningitis based on CSF WBC level and presumed cases were excluded when comparing infected and non-infected patients. Additionally, there was no difference in lactate level of presumed and proven cases. The same methodologic concerns apply to the study of by Maskin et al[90]. In this study the presumed group was included in the infection group and lower WBC cut-offs were used. Tavares et al. used a strict culture-based definition for post operative meningitis, but the study population was small (n=28) with few (n=7) meningitis cases. While lactate was significantly higher in EVDRI patients, both WBC and glucose had higher AUC of ROC[78]. The 2017 study by Grille et al. represents the first assessment of lactate for EVDRI specifically[91]. This study included 36 patients where 14 fulfilled EVDRI criteria. One of the criteria, in addition to positive CSF culture, was CSF WBC >500/μL, which, considering that there is an association between CSF WBC and lactate, would overestimate the diagnostic performance of lactate. This also applies to the study by Li et al. that used WBC >1 000/μL as cut-off and excluded cases with WBC >500/μL from the culture negative group[92].

In a study were CSF from all patients (n=467) that had CSF sampled by an EVD in a tertiary care centre during a five-year period were analysed for lactate, and a strict culture-based definition of EVDRI was used, it was concluded that no cut-off for CSF lactate provided both sensitivity and specificity high enough for it to be regarded as a reliable test[93]. Another study of 90 blood-contaminated CSF samples, where 6 were culture positive, showed a lower diagnostic value of lactate compared to WBC to predict a positive culture result[94]. In their prospective study from 2022, Dorresteijn et al. observed no significant difference in CSF lactate between EVDRI and non-EVDRI patients[85]. Taken together, it does not seem like lactate provides a large improvement over WBC in diagnostic precision for EVDRI.

Other CSF biomarkers

CSF glucose, protein or albumin have been described as unspecific markers for infection[70] and have not been as extensively studied as WBC or lactate. Existing data confirm significant overlap between EVDRI and non-EVDRI groups regarding CSF glucose, CSF/plasma glucose ratio and even more so regarding CSF protein concentration[95,96]. In a review of EVDRI CSF biomarker studies, 6 of 15 included studies on CSF protein and 4 of 10 studies of CSF glucose showed no significant difference between EVDRI and non-EVDRI groups[72].

CSF procalcitonin has also been assessed as a biomarker for PNBM with somewhat disappointing results[92,97,98]. However, it seems to have a weaker association to CSF WBC than other proposed biomarkers which is a theoretical advantage.

Several novel CSF biomarkers for EVDRI have also been suggested, such as interleukin-6 (IL-6) as well as the neutrophil protein heparin-binding protein (HBP)[98–101]. The same methodologic issues with either small materials and/or varying definitions of

infection also apply to these studies. So far, these biomarkers have not been put into widespread clinical use.

Table 2: Examples of studies of CSF biomarkers for diagnosing EVDRI/HCAVM.

Study	Biomarker	Patients (n)	Infections (n)	Infection definition	Result
Pfausler (2004)[81]	Cell-index	13	7	WBC increase, CSF/serum glucose reduction + positive culture	Cell-index higher in EVDRI group
Lunardi (2017)[83]	Cell-index	34	20	CDC based, No positive culture required	Cell-index 2.9: Sens 95%, spec 92.9%, AUC 0.982
Montes (2019)[74]	Cell-index	111	37	Positive culture + CDC criteria	AUC 0.825 for Cell-index (0.653 for WBC)
Liew (2020)[84]	Cell-index	95	7	Positive culture	Cell-index >10.4: Sens 80.5%, spec 70.5% AUC 0.727
Böer (2010)[94]	Lactate	90	6	Positive culture	No significant difference between culture positive and negative samples.
Grille (2017)[91]	Lactate	36	14	Positive culture + WBC >500/μL or glucose<0.5 g/L	Lactate >4.15 mmol/L: Sens 86%, spec 86% AUC 0.90.
Hill (2017)[93]	Lactate	467	22	Positive culture	Lactate >4.0 mmol/L: Sens 72.7%, spec 76.0% AUC 0.82
Schoch (2008)[101]	IL-6	75	20	Clinical symptoms + increased WBC and protein	IL-6 >2.7μg/mL: Sens 73.7%, spec 91.4%
Hopkins (2012)[99]	IL-6	25	6	Positive culture + clinical signs	IL-6 was significantly higher in EVDRI patients. All EVDRI had >10μg/mL
Lenski (2017)[100]	IL-6	63	17	Positive culture or glucose <0.4, WBC >100/μL, or protein >0.5g/L	IL-6 >707pg/mL: Sens 100%, spec 100% AUC 1.00

*Sens=sensitivity, spec=specificity, AUC=Area under ROC-curve.

Diagnosis

Bacterial culture

Current Infectious Diseases Society of America (IDSA) guidelines state that bacterial culture of CSF represents the gold-standard for diagnosing bacterial meningitis or ventriculitis, including EVDRI and other healthcare associated forms of meningitis[102,103]. These guidelines do not specify how CSF cultures should be performed other than that they should be held for 10 days, if initial cultures are negative

and there is a suspicion of infection[103]. Microbiological studies of CSF culture methodology in CNS infections have found significant differences in diagnostic yield depending on the culture methods used at the laboratory, with increased recovery rate of microorganisms with the use of broth cultures, anaerobic cultures, and prolonged culture duration[104–107]. A small unpublished survey of six academic clinical microbiology laboratories in the USA revealed that culture procedures varied widely between laboratories[108]. In Swedish clinical microbiology laboratories, CSF cultures are generally performed on both solid agar plates as well as in broth medium, such as blood culture bottles. However, the exact choice of agar plats, enrichment medium, blood culture system and duration of cultures varies[109].

Polymerase chain reaction methods

Since their introduction in the 1990s, polymerase chain reaction (PCR) methods that amplify and detect specific sequences of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) have become one of the most important tools in the clinical microbiological laboratory. There are commercial multiplex PCR methods for detecting the most common viral, fungal, and bacterial causes of meningitis and encephalitis such as the FilmArray® Meningitis/Encephalitis panel (Bio-Fire Diagnostics) that has been thoroughly assessed in clinical use[110]. This and other commercial PCR panels are developed for the diagnosis of community acquired meningitis and hence, have little or no use in EVDRI or other healthcare associated meningitis diagnosis due to the different spectrum of pathogens encountered in these infections. Instead, the most widely used PCR method for EVDRI is PCR targeting the 16S ribosomal RNA (rRNA) gene. The 16S rRNA gene codes for rRNA of the 16S sub-unit of the bacterial ribosome. This gene is conserved between bacterial species allowing for detection of a wide range of bacteria. However, the exact nucleotide sequence varies between species and hence, allow for species identification by sequencing the PCR-amplified DNA. PCR of the 16S rRNA gene has been reviewed ABM[111], but there is limited scientific evaluation of its performance in EVDRI.

Early studies of 16S PCR on CSF of NIC patients showed that bacteria could be detected with 16S PCR in patients with suspected infection but negative cultures[112,113]. These findings have not been consistently reproduced in later studies where cases with negative CSF culture but positive 16S PCR appear more uncommon[114–116]. However, two recent studies showed numerous cases of PCR positivity in culture negative samples suggesting 16S PCR could increase detection of EVDRI and PNBM[117,118]. Using new metagenomic Nanopore technology for 16S-sequencing, Jang et al. demonstrated an increased detection of bacterial meningitis after neurosurgery as 23 of the 41 episodes (56.1%) were diagnosed by 16S PCR despite negative culture[119]. Despite differences in PCR positivity rates in culture negative cases, the studies on 16S PCR have generally shown good concordance of PCR and culture in culture positive cases, except for the study of Zarrouk et al., where only 2 of 6 culture positive subjects were positive in 16S

PCR[116]. Table 3 summarise the characteristics and results of studies of the use of PCR for EVDRI and PNBM diagnosis.

Table 3: Characteristics and main results of studies of PCR for EVDRI or PNBM diagnosis.

Study	Patients/ samples (n)	Infections (n)	Infection definition	Result
Druel (1996)[112]	57/57	27 (20 culture negative)	WBC >100/μL + RBC/WBC-ratio < 200	20/20 PCR tested patients in meningitis group PCR positive. No samples PCR positive in controls.
Banks (2005)[113]	28/86	N/A	N/A	18/18 culture positive samples were PCR positive (100%). 25 of 68 culture negative samples PCR negative (37%).
Deutch (2007)[114]	86/350	N/A	N/A	16/34 culture positive samples were PCR positive (47%). 21/316 culture negative samples were PCR negative (93%).
Zarrouk (2010)[120]	32	6	Positive culture + WBC >100/μL	2/6 culture positive patients were PCR positive (33%). 26/26 culture negative patients were PCR negative (100%).
Rath (2014)[115]	42/62	42	At least 2 of: clinical symtoms, 100% WBC rise, pos culture/PCR	17/21 culture positive samples were PCR positive* (81%). 40/41 culture negative samples were PCR negative* (98%).
Gordon (2015)[121]	45/45	9	Positive culture + clinical signs	6/9 culture positive patients were PCR positive** (67%). 25/36 culture negative patients were negative in PCR** (69%).
Dąbrowski (2017)[118]	50/276	8/12 (By culture /PCR)	Positive culture/PCR + clinical signs	PCR identified 4 additional infections. 82% of positive cultures in the infection group were PCR positive.
Perdigão (2021)[117]	51/51	4	Positive culture	3/4 culture positive patients were PCR positive (75%). 26/47 culture negative samples were PCR negative (55%).
Jang (2022)[119]	178/285	41	Positive culture and PCR result or discordant PCR and cultue result + case assesment	23/41 “genuine infection” samples were only positive in PCR. 17/22 culture positive were PCR positive (77%). 240/263 culture negative samples were PCR negative (91%).

*Using the SeptiFast (Roche Molecular Diagnostics) multiplex PCR panel.
**Using self-developed multiplex PCR panel.

Considering the limited evidence, the 2017 IDSA Clinical Practice Guidelines for Healthcare-Associated Ventriculitis and Meningitis state that more studies are needed before routine use of PCR can be recommended in this setting[103]. Despite this, 16S PCR is used clinically to diagnose EVDRI, and a recently published study illustrated that it could reduce the use of antimicrobials to treat patients with suspected EVDRI

with negative CSF cultures[122]. Of course, this depends on whether clinicians trust the PCR results.

Definitions

There are no universally adopted definitions of EVDRI which represents a major problem both scientifically and clinically. This was recognized by Lozier et al. in their review from 2002 where they suggested definitions of confirmed and suspected EVDRI as well as contamination and colonisation, summarized in table 4[34].

Table 4: Sugested definitions by Lozier et al.
Adapted from Lozier AP, Sciacca RR, Romagnoli MF, Connolly ES Jr. Ventriculostomy-related infections: a critical review of the literature. Neurosurgery. 2002 Jul;51(1):170-81.

Term	Definition
Contamination	Isolated positive CSF culture and/or Gram's stain
	Expected CSF glucose and protein profile
	Expected CSF cell count
Colonisation	Multiple positive CSF cultures and/or Gram's stains
	Expected CSF profile
	Expected cell count
	Lack of clinical symptoms other than fever
Suspected EVDRI	Progressively declining CSF glucose level
	Increasing CSF protein profiles
	Advancing CSF pleocytosis
	Absence of positive CSF cultures or Gram's stains
EVDRI/VRI	Progressively declining CSF glucose level
	Increasing CSF protein profiles
	Advancing CSF pleocytosis
	One or more positive CSF culture or Gram's stain
	Paucity of clinical symptoms other than fever
Ventriculitis	Low CSF glucose level
	High CSF protein
	CSF pleocytosis
	Fever
	Clinical signs of meningitis, including nuchal rigidity, photophobia,
	decreased mental status, seizures, or moribund appearance

Despite these efforts, varying definitions have remained, which is illustrated by a study from 2016 that managed to find 16 different EVDRI definitions when reviewing the literature. When applying these definitions to a test cohort of patients with confirmed EVDRI, EVDRI incidences ranged from 22-94%[123].

The most widely referenced definitions are the Centre for Disease Control and Prevention's National Healthcare Safety Network (CDC/NHSN) definitions that define many types of infections, including meningitis and ventriculitis which are also adopted to EVDRI specifically[124]. The CDC criteria are summarised in the table below.

Table 5: CDC/NHSN diagnostic criteria for meningitis or ventriculitis.

For the diagnosis of ventriculitis or meningitis one of following has to be fulfilled
1. Patient has organism(s) identified from cerebrospinal fluid (CSF) by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment for example, not Active Surveillance Culture/Testing (ASC/AST).
2. Patient has at least *two* of the following: i. Fever (>38.0°C) or headache ii. Meningeal sign(s)* iii. Cranial nerve signs* And at least *one* of the following: a. Increased white cells, elevated protein, and decreased glucose in CSF (per reporting laboratory's reference range). b. Organism(s) seen on Gram stain of CSF. c. Organism(s) identified from blood by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment, for example, not Active Surveillance Culture/Testing (ASC/AST). d. Diagnostic single antibody titer (IgM) or 4-fold increase in paired sera (IgG) for organism **With no other recognised cause*

A problem with the CDC criteria is that they are not adapted to the NIC setting. Criterion 2 allows for the diagnosis without a positive microbiological test result of CSF. Also, criterion 2a refers to local laboratory reference ranges regarding cut-offs for what should be interpreted as increased or decreased in CSF parameters. Considering that abnormal CSF cytochemistry is very common in NIC patients this allows for liberal diagnosis of ventriculitis/EVDRI if standard clinical cut-offs are used.

The 2017 IDSA clinical practice guidelines for the management of healthcare-associated ventriculitis and meningitis do not specify strict criteria for EVDRI diagnosis but include some recommendations regarding the diagnosis[103]. These include:

- Abnormalities of CSF cell count, glucose, and/or protein may not be reliable indicators for the presence of infection in patients with healthcare-associated ventriculitis and meningitis.
- CSF culture is the most important test to establish the diagnosis of healthcare-associated ventriculitis and meningitis.
- CSF pleocytosis with a positive culture and symptoms of infection are indicative of a diagnosis of healthcare-associated ventriculitis or meningitis.
- Growth of an organism that is commonly considered a contaminant (e.g. coagulase-negative staphylococci) in enrichment broth only or on just 1 of multiple cultures in a patient with normal CSF and no fever is not indicative of healthcare-associated ventriculitis or meningitis.
- CSF cultures that grow *Staphylococcus aureus*, aerobic gram-negative bacilli or fungal pathogens are indicative of infection.

These recommendations are hard to implement into definitions but reflect that CDC/NHSN definitions must be adjusted in the NIC/EVDRI setting. Guidelines from the Swedish Infectious Diseases Society (SILF) conclude that abnormal CSF cytochemistry can suggest infection, that CSF culture of *Staphylococcus aureus*, gram-negative bacteria, streptococci, or enterococci are strongly indicative of infection and that CSF culture with coagulase-negative staphylococcus (CoNS) or *Cutibacterium acnes* may represent contamination, and repeated findings of the same pathogen increase the likelihood of infection[125].

As established definitions are lacking, researchers have used their own definitions in their research. These can be broadly structured into the following categories:

1. Definitions requiring no positive CSF microbiological test result, solely CSF cytochemistry abnormalities with or without clinical symptoms is defined as infection.
2. Definitions requiring positive CSF microbiological test results. These can be further divided into the following categories:
    a. Positive CSF microbiology alone is defined as infection.
    b. Positive CSF microbiology with certain pathogens (for example *Staphylococcus aureus* or gram-negatives) alone is defined as infection. Clinical and/or cytochemical signs or repeated positive cultures are required for other pathogens (for example CoNS).

c. Positive CSF microbiology in combination with abnormal CSF cytochemistry is required for EVDRI diagnosis.

It could be argued that category 2c represents the most robust approach, however there is a risk that this approach misclassifies patients with positive CSF microbiology as non-infection due to limited CSF cytochemistry abnormalities. This risk is more pronounced in patients with low-virulent pathogens but also in cases where CSF sampling was performed early in the course of infection, as CSF abnormalities may take time to develop. Another potential risk with definitions requiring positive microbiology is that the microbiological tests may be falsely negative due to limited sensitivity of culture, especially in patients with antimicrobial treatment at the time of sampling.

There is considerable heterogeneity regarding cut-offs for CSF cytochemistry parameters and which CSF cytochemistry parameters that are used to define EVDRI. This applies to definitions in category 1 as well as 2b and 2c. WBC is the most widely included parameter in definitions. Sundbärg et al. used a modest cut-off for WBC of 11/μL to separate infection from contamination in culture positive cases[47], whereas some studies have used WBC of up to 500/μL as an additional criterion for culture positive cases [91]. Values of up to 1 000/μL have been used as criteria in culture negative cases[89]. The SILF guidelines state that the following parameters can support the presence of EVDRI:

Table 6: CSF parameters indicating infection according to SILF guidelines.

CSF parameter	Value indicating infection
WBC	>250/μL with polymorphonuclear domminance or marked increase from previous sample
Lactate	>4.0 mmol/L
Glucose	<2.8 mmol/L
CSF/plasma glucose ratio	<0.35

These cut-offs are largely based on study from 1999 by Leib et al. where these values were used as definition but were not specifically assessed[89]. It should be noted though, that the WBC cut-off of 250/μL in this study is an arbitrary value based on the authors' experiences.

Microbiological aetiology

Not all EVDRI studies report the aetiology in microbiologically positive cases. When reported, CoNS, including *Staphylococcus epidermidis,* is usually the dominating aetiology[34,35]. Among non-CoNS gram-positive bacteria, *Staphylococcus aureus* is the most common cause, but alfa-haemolytic streptococci and enterococci are also reported. Among gram-negative bacteria, the incidence is usually more evenly

distributed between *Enterobacter* spp., *Acinetobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., and other species. The classical ABM pathogens *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* and *Listeria monocytogenes* are never or very seldom seen in EVDRI. When compiling data on bacterial aetiology from 25 studies with 523 positive CSF cultures Ramanan et al. found 64% of infections were caused by gram positives, were CoNS and *S. aureus* accounted for 38% and 15%, respectively. Gram-negative bacteria were isolated in 35% of cases, with *Acinetobacter* spp. representing the most common finding at 9%[35]. However, the spectrum of bacterial aetiology varies greatly by location, illustrated by studies showing a strong gram-positive dominance[41,47,48,69,126,127] and others showing gram-negative dominancee[37,46,128–131].

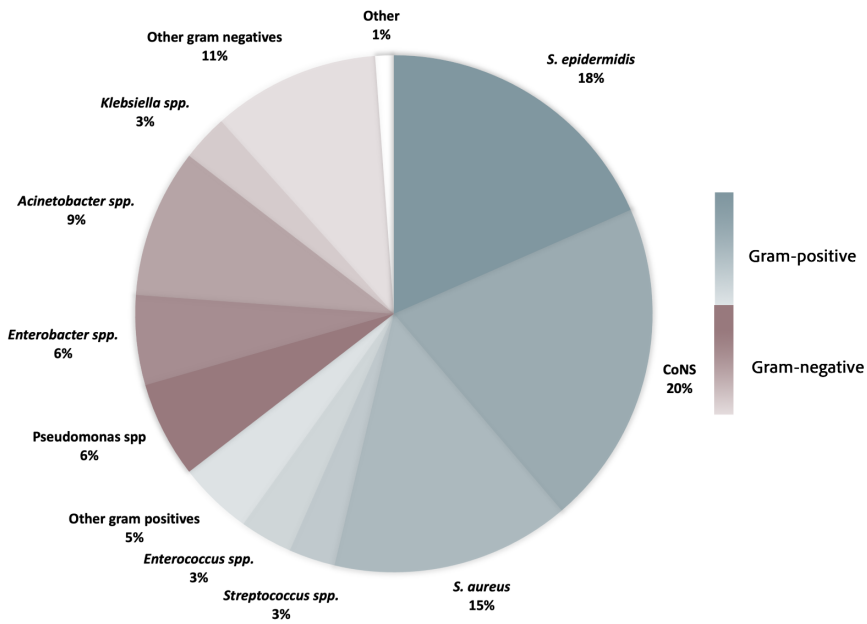

Figure 5: Microbiological aetiology in 516 positive CSF cultures fron 25 studies of EVDRI.
Figure adapted from: Ramanan M, Lipman J, Shorr A, Shankar A. A meta-analysis of ventriculostomy-associated cerebrospinal fluid infections. BMC Infect Dis. 2015 Jan 8;15:3. Copyright © Ramanan et al..; licensee BioMed Central. 2015. Original work licenced under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

Cutibacterium acnes is a gram-positive anaerobic bacteria skin bacteria that has been shown to cause infections of permanent CSF shunts[132,133] and has also been proposed as an important pathogen in EVDRI, however findings of *C. acnes* seem rare in EVDRI. One study detected *C. acnes* in 15% of EVDRI cases[76], but this represents an exception.

Nevertheless, *C. acnes* is difficult to culture, and it could be speculated that it is a more common cause of EVDRI than culture results suggest.

Prevention

Prophylactic antibiotics

Prophylactic systemic antibiotic treatment is widely used to prevent EVDRI but is not universally adopted and regimens vary[35,134]. The 2017 IDSA guidelines for healthcare-associated ventriculitis recommend using periprocedural antibiotics at EVD-insertion while they advise against prolonged prophylactic treatment while the EVD is in place[103]. The same recommendation is found in a consensus statement from the Neurocritical Care Society[135]. The scientific support for prophylactic antibiotics is not unanimous and many studies have only compared periprocedural prophylaxis to prophylactic treatment for the duration of EVD treatment.

Regarding periprocedural prophylaxis, evidence from implantation of permanent CSF shunts, where it has clearly been shown to reduce the risk of infection[136], has been extrapolated to EVD insertion. However, randomized control studies are lacking and since periprocedural antibiotic is standard of care in most centres, it is unsure whether such studies will be performed.

For prolonged antibiotic prophylaxis during the entirety of EVD-treatment, results are conflicting. An early study by Wyler and Kelly showed a reduction in infection incidence from 27% to 9% with the use of prolonged prophylaxis[33], however, it seems unlikely that these results could be applied to modern circumstances. One randomized study has demonstrated a reduced incidence of EVDRI with the use of prolonged compared to periprocedural antibiotic prophylaxis, but when infections did develop, they were caused by more antimicrobial-resistant pathogens, such as Methicillin resistant *Staphylococcus aureus* (MRSA) and *Candida* spp.[137]. Other retrospective studies have shown no benefit of prolonged prophylaxis[36,138,139], and in the study of Dellit et al., there was a statistically significant increase in the risk of acquiring infection with *Clostridioides dificile* in the group with prolonged prophylaxis[138].

Antibiotic impregnated catheters

Antibiotic impregnated (AI) EVD catheters is in clinical use in many centres to reduce the risk of EVDRI and they are recommended in the 2017 IDSA guidelines[103]. However, evidence of their efficacy is not consistent, and their potential side-effects have not been sufficiently studied. Swedish guidelines recommend not using these devices[125]. Catheters are impregnated with Rifampicin and Clindamycin (Bactiseal™) or Rifampicin and Minocycline (VentriClear™). The first randomized controlled trial (RCT) by Zabramski et al. showed a statistically significant difference in EVDRI

incidence from 9.4% in the control group to 1.3% in patients with AI catheters[140]. This is contrasted by a later RCT, with a lower baseline incidence, showing no significant difference in EVDRI incidence between patients with standard and AI catheters[141]. A meta-analysis pooling the results of these studies along with one additional RCT with silver coated catheters still showed a significant risk reduction with the use of coated catheters with a calculated OR of 0.49 (95% CI 0.27-0.89)[142]. A large prospective observational study by Harrop et al. saw a significant decrease in EVDRI when AI catheters were introduced, an increase when they were discontinued, followed by another decrease when reintroduced[143]. There have been concerns that the use of AI catheters could lead to increasing rates of more antibiotic-resistant pathogens, but data so far is limited and not consistent[144].

Silver coated catheters

The antimicrobial effect of silver is well known and there are several examples of medical devices coated with silver to reduce the risk of infection, including silver coated (SC) EVD catheters. An RCT of Keong et al. showed a statistically significant reduction in the incidence of EVDRI in the intervention group with SC EVDs to 12.3%, compared to 21.4% in the control group[145]. This is contrasted by a large prospective multicentre cohort study that found no difference in EVDRI incidence between patients with standard-, AI- and SC catheters[63] as well as meta-analyses pooling results of observational studies[142,146]. The 2017 IDSA guidelines do not mention SC catheters specifically and Swedish guidelines recommend against their use[103,125].

Routine CSF sampling

As clinical symptoms of EVDRI are unspecific and hard to detect in unconscious patients, routine CSF sampling for cytochemistry and/or culture at specified intervals has been employed to diagnose EVDRI at an early stage. Different frequencies ranging from daily to weekly sampling has been proposed. However, frequent sampling has often been associated with an increased risk of EVDRI[38,42,63]. While this might not be a causal relationship, the association was persistent in a multi-variate analysis in one study[147]. There are also studies questioning the value of routine CSF sampling[96,148] and others showing benefits of reducing the frequency of sampling[42]. Given the limitations of CSF biomarkers described earlier, as well as the risk of false positive cultures due to contamination, it seems obvious that there is a risk that routine CSF sampling can cause diagnostic difficulties and result in unnecessary antibiotic treatment regardless of whether the sampling itself affect the risk of EVDRI.

Catheter exchange

In a frequently cited study from 1984 Mayhall et al. noted an association between EVD treatment for more than five days and EVDRI and, hence, recommended that EVD catheters should be replaced every five days[45]. This led to the implementation of this

practice in many centres. However, routine exchange of EVD catheters has later been questioned and instead, associated with increased risk of infection[39,49,60], and is not recommended by the Neurocritical Care Society[135].

EVD handling

While less studied and perhaps harder to isolate, factors in daily clinical EVD handling and maintenance most likely influence EVDRI incidence. There are several studies showing reductions in EVDRI incidence upon implementation of so-called bundles of measures regarding EVD placement, draping, handling, and sampling[57,130,149–153]. As the bundles were different in different studies and some involved the introduction of AI catheters or antibiotic prophylaxis, it is hard to isolate which measures are most effective, but the results illustrate the importance of routines regarding daily EVD handling performed by nursing staff.

Treatment

Empirical treatment

Since results of bacterial cultures take several days, empirical antibiotic treatment is often initiated in EVD patients with pathologic CSF cytochemistry. The regimen of empirical treatment varies by location, based on the local microbiological spectrum, antibiotic availability, and clinical tradition. Generally, systemic treatment with intravenous antibiotics is used and it should have a broad effect on both gram-positive and gram-negative bacteria as well as sufficient CNS penetration to result in adequate CSF concentrations. This means combination therapy with vancomycin and a beta-lactam with gram-negative coverage is usually prescribed.

Vancomycin has long been the standard choice for gram-positive coverage and is recommended by both IDSA and SILF guidelines[103,125]. However, the latest revision of SILF guidelines promote linezolid as an alternative for gram-positive coverage. Linezolid has shown excellent penetration to CNS which has also been confirmed in neurosurgical patients[154–156]. One small study comparing linezolid to vancomycin treatment in patients with ABM caused by MRSA showed an increased rate of bacterial clearance of CSF on day five in patients treated with linezolid[157]. Another small observational study showed bacterial clearance on day five of Linezolid treatment in 8/8 patients with post neurosurgical meningitis or ventriculitis caused by methicillin resistant staphylococci[158].

Regarding the choice of beta-lactam, the IDSA guidelines advice using a drug with anti-pseudomonal activity such as cefepime, ceftazidime or meropenem[103]. Given the low frequency of *Pseudomonas* spp. and ESBL-producing enterobacterales in the Scandinavian setting, SILF guidelines recommend cefotaxime as the first line choice of

beta-lactam and meropenem if there is increased risk of a resistant bacteria or a poor response to initial treatment[125].

Discontinuation of empirical treatment

As CSF cultures are often negative, but usually held for 7-14 days, the timing of discontinuation of empirical treatment when cultures remain negative has been subject of debate. A consensus document from 2000 by the British Society for Antimicrobial Chemotherapy recommended treatment withdrawal if cultures were negative after 48–72 hours in postoperative meningitis[159]. This recommendation was based on clinical experience of the authors and not validated in clinical trials. A later cohort study attempted to evaluate this practice by comparing outcomes in patients with postoperative meningitis before and after the implementation of antibiotic discontinuation in subjects with negative culture after 3 days. The authors found that all cases of both bacterial and aseptic meningitis resolved while mean duration of antibiotic treatment of culture negative cases was reduced from 11 to 3.5 days[120]. It should be noted that this study was not performed on patients with an EVD. One of few studies reporting time to CSF culture positivity in EVDRI reported a median time to culture positivity of 3 days[85]. If this is representative, it would mean discontinuation on day three would leave many patients with longer time to culture positivity without treatment after three days. Neither IDSA or SILF guidelines give advice on when to discontinue empirical antibiotic treatment, but SILF guidelines state that empirical treatment should be withheld for at least 10 days if there is a strong clinical suspicion of EVDRI or shunt infection, despite negative culture and/or PCR results[125].

Directed therapy

Once a pathogen has been isolated in CSF culture or identified by PCR, treatment is directed based on susceptibility testing from the culture or by local resistance patterns of the identified bacteria in cases detected with PCR. Initially, this usually results in continued therapy with one of the empirical antibiotics. In gram-positive infections, vancomycin or linezolid is continued while the Beta-lactam is discontinued and vice versa for gram-negative infections. Other antimicrobials can also be used after susceptibility testing, including trimetoprim/sulfametoxazole, quinolones, rifampicin or rifampin, fosfomycin, penicillin G and amphotericin B, flucytocine or azoles in the rare event of a fungal infection.

Rifampicin and rifampin are unique in the fact that they have been shown to be effective against bacteria in biofilm[160]. As this is probably an important factor in neurosurgical infections with indwelling hardware such as shunts and EVDs, the IDSA guidelines recommend combination therapy with rifampicin in staphylococcal infections in patients with implanted material, including EVDs[103]. SILF guidelines,

however, advice against rifampicin combination therapy in EVDRI due to the temporary and relatively short nature of EVD-treatment[125].

Intraventricular antimicrobial therapy

As sufficient CSF concentrations can be hard to achieve, especially with vancomycin due to its limited CNS penetration and toxicity in higher intravenous dosing, local antimicrobial therapy administered through the EVD has been used. Although this route of administration is not approved by medical regulatory authorities, it was proposed for vancomycin by the British Society for Antimicrobial Chemotherapy in 2000 after several years of clinical use in various institutions[159]. A systematic review of subsequent studies of intraventricular vancomycin therapy, mainly comprised of case reports, case series and one prospective randomized study concluded intraventricular vancomycin treatment appeared safe and effective[161].

For gram-negative infections, intraventricular vancomycin is not effective due to its antimicrobial spectrum. As beta-lactams can be neurotoxic in high concentrations they cannot be used for intraventricular treatment. Instead, intraventricular treatment with aminoglycosides has been used. Gentamicin has been the most used drug for intraventricular use, but tobramycin and amikacin treatment has also been described. Intraventricular treatment with aminoglycosides has been assessed as safe and effective in a review of 18 published studies[162]. Notably, one retrospective Swedish study of 31 patients, where 18 were treated with intravenous antibiotics alone and 13 with the addition of intraventricular gentamicin, showed no relapses in the combination-therapy group and 33% risk of relapse in the control group[163].

Use of other antimicrobials, such as daptomycin, teicoplanin and colistin, for intraventricular treatment has been described but should be considered experimental and reserved for infections with no other effective treatment options[164–166].

Consequences of EVDRI

In the literature, EVDRI is often described as a serious condition associated with considerable morbidity and mortality. However, references to scientific assessments of its consequences are usually lacking. While ABM is clearly associated with substantial morbidity and mortality[167], this cannot be extrapolated to bacterial meningitis or ventriculitis due to an EVD catheter, as this represents an altogether separate disease entity. Still, EVDRI can sometimes cause marked symptoms and CNS inflammation, and it seems reasonable to assume that it can worsen the outcome of NIC patients.

Outcomes after NIC of TBI and SAH patients are usually reported in either the Glasgow outcome scale (GOS) [168], its extended version (GOSE)[169] or the modified Rankin scale (mRS) [170,171]. Details of the GOS, GOSE and mRAS is listed in Table 7.

Table 7: Details of scales used for assessing outcome after NIC

	GOS	GOSE	mRAS
0	-	-	No symtoms
1	Death	Death	No significant disability
2	Persistent vegetative state	Persistent vegetative state	Slight disability
3	Severe disability	Lower severe disability	Moderate disability
4	Moderate disability	Upper severe disability	Moderate severe disability
5	Good recovery	Lower moderate disability	Severe disability
6	-	Upper moderate disability	Death
7	-	Lower good recovery	-
8	-	Upper good recovery	-

As the outcome after NIC is extensively multi-factorial, underlying conditions have high morbidity and mortality, and the outcome measures described above are somewhat blunt, it is challenging to study the isolated impact of EVDRI on outcome. In a recent meta-analysis of studies reporting mortality of EVDRI patients and controls, only 2 of 12 included studies reported a significantly higher mortality in the EVDRI group and the pooled morality was not significantly higher in subjects with EVDRI with an OR for death of 1.07 (95% CI 0.59–1.92)[172]. However, it should be noted that the largest study included in the meta-analysis, which used registry data from 34 000 patients, showed a significantly higher OR for death in patients with EVDRI after controlling for confounding factors (adjusted OR 1.38, 95% CI 1.22–1.46)[173]. Functional outcome was less frequently reported in the studies included in the meta-analysis, and heterogeneity in reporting reduced comparability, but only one of five studies with data reported worse functional outcomes in the EVDRI group[131] and the authors of the meta-analysis concluded EVDRI is not associated to increased mortality or worse functional outcome. There was, however, a strong association of EVDRI and increased EVD duration and increased length of ICU and hospital stay[172]. This leads to both personal suffering for the affected patients and to increased healthcare costs. The latter has been quantified in a retrospective study where total healthcare costs were estimated to be twice as high in NIC patients with EVDRI, compared to non-EVDRI patients[174] as well as in a large registry-based study with similar results[173].

Aims

The overall aims of the papers included in this thesis were:

I. To study the incidence and microbiological aetiology of EVDRI in Swedish SAH patients.

II. To study the extent of antimicrobial therapy for suspected and confirmed EVDRI in Swedish SAH patients.

III. To investigate the diagnostic performance of Heparin-binding protein in CSF as a biomarker for EVDRI.

IV. To investigate the performance of 16S PCR-based diagnostics for EVDRI.

V. To characterise the CSF proteome in EVDRI and, if possible, identify potential CSF biomarkers for EVDRI by mass spectrometry.

Material and methods

Study design

	Paper I	Paper II	Paper III	Paper IV
Study design	Observational, descriptive	Observational, analytic	Observational, analytic	Observational, analytic
Inclusion	Retrospective, consecutive	Prospective, consecutive	Prospective, non-consecutive	Prospective, non-consecutive
Populatiom	SAH patients treated with EVD at Uppsala university hospital between 2010–2013 (n=191)	Neuro-ICU patients treated with EVD at Lund university hospital Jan 2009–Mar 2010 (n=90)	Neuro-ICU patients >18 years with EVD and suspicion of EVDRI at CSF sampling (n=84)	Neuro-ICU patients >18 years with EVD and ABM or suspicion of EVDRI at CSF sampling (n=92)
EVDRI-definition	Positive CSF culture and corrected* CSF PMNC > 50/μL or CSF lactate > 3.5 mmol/L or CSF/plasma glucose ratio < 0.4 or CSF albumin > 0.4 g/L	Positive CSF culture or 16S PCR + corrected* CSF WBC > 50/μL	Positve CSF culture Clinically assesed as EVDRI if antimicrobial therapy against the cultured bacteria in CNS doseing was prescribed for ≥ 7 days.	Positve CSF culture
Experimental procedures	None	HBP ELISA on all CSF samples	Real-time 16S PCR and 16S PCR with Nanopore sequencing on selected CSF samples.	Liquid chromatography tandem mass spectrometry (LC-MS/MS) on all CSF samples.
Main outcomes	EVDRI incidence, microbiological aetiology and prescribed EVDRI treatment.	Sensitivity, specificity, PPV, NPV, ROC AUC for HBP. Median HBP for diagnosis groups. EVDRI incidence and microbiological aetiology.	Sensitivity and specificity for CSF 16S PCR in relation to CSF culture. Comparison of 16S sequence numbers between culture positive and culture negative subjects +/- antimicrobial therapy at sampling.	Differentialy expressed proteins in CSF when comparing EVDRI and ABM subjects to subjects without signs of infection.

* Calculated as CSF PMNC or WBC - CSF-erythrocytes/1000

Study populations

For paper I, the study population was obtained through screening of medical records of patients in a prospectively collected clinical database of consecutive patients with non-traumatic SAH admitted to the neuro-ICU of Uppsala University Hospital that had been treated with an EVD between 2010–2013. Patients that received substantial parts of the neuro-ICU care at another hospital were excluded, as well as patients whose clinical management considering CSF sampling were affected by another clinical study. This resulted in a study population of 191 subjects.

In paper II, patients treated with an EVD at the neuro-ICU of Lund University Hospital, that had at least one CSF sample drawn from the EVD, were consecutively included between January 2009 and March 2010. Patients considered to have a pre-existing CNS infection (ABM or shunt infection) at admission were excluded, resulting in 90 included subjects.

For paper III and IV a prospectively collected cohort of patients >18 years treated with an EVD at the neuro ICU or neuro-intermediary care unit (neuro-IMCU) at Uppsala University Hospital between February 2018 and November 2021 was used. Patients were included when CSF was sampled from the EVD and sent for bacterial culture due to clinical suspicion of CNS infection. Inclusion was not consecutive, as it was at times reduced or paused due to capacity issues. When inclusion of culture negative subjects met the pre-allocated number, only culture positive subjects were included. For paper III, subjects were divided into one of three study groups based on the result of the CSF culture and, for culture negative subjects, whether the CSF sample was collected during antibiotic treatment or not. To reduce challenges in group allocation, CSF culture negative subjects with a positive CSF culture prior to inclusion were excluded as well as subjects where the indication for EVD placement was ABM or shunt-infection. Subjects with ABM were, however, included in paper IV as a separate group. This resulted in study populations of 84 and 92 patients in paper III and IV, respectively.

CSF sampling policy

At the period of inclusion of the subjects in paper I, III and IV, CSF sampling was only performed at clinical indication, as judged by the treating physician and supporting infectious diseases specialist. During the inclusion of subjects in paper II, CSF sampling for cytochemistry and culture was performed routinely twice per week on all EVD

patients. However, additional sampling could also be performed on clinical indication, mainly suspicion of EVDRI or to assess the effect of EVDRI treatment.

EVDRI definitions

In paper I, EVDRI was defined based on CSF culture result and local management guidelines regarding interpretation of CSF cytochemistry in EVD patients at Uppsala University Hospital. For EVDRI diagnosis a positive CSF culture was required as well as at least one of the following cytochemical criteria in the same CSF sample:

- Corrected* CSF polynuclear WBC >50/μL
- CSF lactate >3.5 mmol/L
- CSF/plasma glucose ratio <0.4
- CSF albumin >0.40 g/l

*Calculated as CSF polymorphonuclear WBC - CSF-erythrocytes/1000

In paper II, the definition was slightly altered as data on CSF lactate and albumin was not available, as these analyses were not routinely performed at the time of the study inclusion. For EVDRI diagnosis a positive CSF culture was required as well as a RBC-corrected CSF WBC of >50/μL in the same CSF sample.

In paper III and IV. Only subjects where CSF sampling was performed due to clinical suspicion of infection were included and all subjects with positive CSF culture were included in the culture positive/EVDRI group. In paper III, however, it was also assessed whether the CSF culture finding was regarded as relevant by the treating physicians, based on prescribed antimicrobial treatment.

Statistical methods

In paper I Mann-Whitney U test and Fisher's exact test were used for group-wise comparisons as appropriate. For paper II and III Mann-Whittney U-test was used for group-wise comparisons. Spearman's rank correlation was used to assess the correlation between CSF biomarkers in paper II. In paper IV, students t-test with Benjamini-Hochberg correction was used to compare expression of individual proteins between groups.

Experimental procedures

HBP analysis

HBP concentration was determined by enzyme-linked immunosorbent assay (ELISA). Briefly. microtiter plates were coated with a mouse monoclonal antibody directed against human HBP. Plates were washed with phosphate buffered saline containing 0.05% Tween and blocked with 2% bovine serum albumin. CSF samples were diluted 1:40 in sample buffer (1 M NaCl) and was added to the wells in duplicate and incubated for 60 min at 37°C. After being washed, the plates were incubated with a polyclonal rabbit antiserum toward human HBP diluted 1:7000. Bound antibodies were detected by incubation with peroxidase-conjugated antibody against rabbit immunoglobulin G in 1:3000 dilution. After plates were developed, the optical density at 405 nm was determined. Finally, the sample HBP concentration was determined by correlating the mean optical densities of the duplicates to the results from a standard curve.

16S rRNA gene analysis

Principle

The 16S ribosomal ribonucleic acid (rRNA) gene is one of several rRNA genes in the genome of prokaryotic cells whose DNA sequence codes for RNA that constitute parts of the ribosome. Specifically, 16S rRNA is a part of the 30S subunit of the ribosome and has both structural and functional roles in the ribosome. The 16S rRNA gene is approximately 1500 nucleotides long, although the exact length varies between species. The gene contains both regions that are variable, named V1-V9, as well as conserved between different prokaryotes. This allows the design of pan-bacterial PCR primers that bind to the conserved regions, while sequencing of more variable regions between the primer binding sites can serve to identify the specific organism present in a sample[175].

As PCR of long DNA-sequences has been associated with a lower sensitivity and higher risk of PCR failure, the standard approach to clinical 16S PCR diagnostics has been to target selected parts of the 16S rRNA gene. These usually contain some of the V1-V4 regions. In the event of a positive PCR reaction, Sanger sequencing is performed to establish the nucleotide sequence of the amplicon. However, only targeting parts of the gene limits the ability to discriminate between closely related organisms. Also, Sanger sequencing is labour intensive and is problematic in the event of a polymicrobial infection[176].

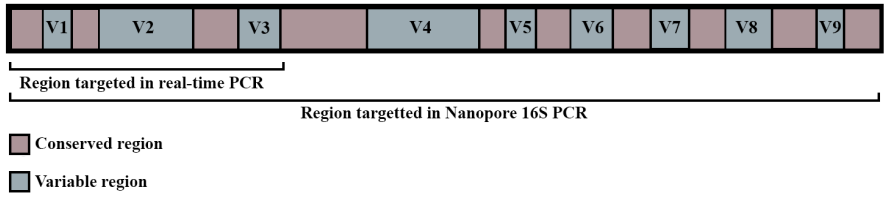

Figure 6: **Schematic illustration of the 16S rRNA gene.**
The location of its variable regions (V1–V9) is shown, as well as which parts of the gene that were targeted in the PCR reactions in paper III.

New technologies for nucleotide sequencing, termed Next-generation sequencing (NGS), have greatly improved nucleotide sequencing performance[177]. One example of NGS is Nanopore sequencing, which is called a third-generation sequencing technology. Nanopore sequencing utilizes nanopores, proteins originally encountered in bacteria, that have properties that allow DNA strands to pass through them. Multiple nanopores are placed in a membrane in what is called a flow cell. A voltage is applied over the membrane, creating an ionic current through the nanopores. As DNA strands pass through the nanopores, the current is altered depending on which nucleotide bases that are passing through the nanopore. By measuring the alterations of the current, the DNA sequence of each strand can be established. As several thousand nanopores can be embedded in the membrane of a flow cell and each nanopore can sequence up to 450 nucleotides per second, rapid sequencing of large amounts of DNA is possible. Moreover, this technology allows for sequencing of long DNA strands of several million bases[178].

Long-read sequencing makes it possible to sequence the 16S rRNA gene in its entirety, allowing for more detailed discrimination of different microorganisms. Furthermore, the fact that DNA strands are sequenced individually means that polymicrobial infections can be diagnosed and DNA sequences from different microorganisms can be quantified separately[179].

Procedure

All PCR experiments in paper III were performed at the Department of Laboratory Medicine, Clinical Microbiology at Örebro University Hospital. Briefly, extraction was performed with the MagDEA Dx kit on MagLEAD 12gC (Precision System Science Co., Ltd., Chiba, Japan) after the samples had been pre-treated with mutanolysin.

A Lightcycler 2.0 system (Roche Diagnostics, Mannheim, Germany) was used for the real-time PCR. The primers used target the first 500 base-pairs of the 16S gene, containing the V1-V3 regions[180]. Each PCR run included a positive PCR control (DNA from *Streptococcus pneumoniae*) and the negative extraction control. Samples with a crossing point (Cp) value at least 1.5 cycles lower than the negative extraction control were considered positive.

For the 16S nanopore sequencing, 16S Barcoding kit 1-24, SQK-16S024 (Oxford Nanopore Technologies, Oxford, England), including primers targeting the entire 16S rRNA gene, were used for library preparation. Based on method development on mock bacterial colonies, the manufacturer's protocol was slightly modified to increase sensitivity. A lowered annealing temperature was used and the number of thermocycles was increased from 25 to 40, which has been described in previous studies on clinical samples[181]. PCR amplification was performed on a VeritiTM Thermal Cycler (ThermoFischer Scientific, Waltham, MA, USA). DNA content in samples after PCR was assessed using a Qubit fluorometer (ThermoFisher Scientific) and 0.5-2.0 µL (depending on DNA content) from each sample was pooled for the sequencing. Sequencing was run for 12 h on a R9.4.1 flow cell in a GridION instrument (Oxford Nanopore Technologies) using Super-accurate basecalling. The 1928 platform (1928 Diagnostics, Gothenburg, Sweden) was used for taxonomic classification. All sequencing reads with a length corresponding to the 16S rRNA gene were mapped against the SILVA (v138.1) reference database for taxonomic assignment[182]. Up to 100 000 reads were used for taxonomic classification.

Mass spectrometry

Principle

For paper IV, the proteome of CSF samples was analysed with liquid chromatography tandem mass spectrometry (LC-MS/MS). This means that, after initial sample preparation including protein enrichment and enzymatic cleavage of proteins to peptides, the sample was passed through a liquid chromatography (LC) device. A liquid chromatography device contains a column with a stationary phase, or adsorbent, through which a mobile phase containing the sample and solvent is pushed with high pressure. The time required for the different molecules in the sample to pass through the column differs depending on their chemical properties, which affect their interactions with the solid phase. This means they are separated and exit the LC column at different times[183]. After separation by LC, the peptides are introduced into the mass spectrometer.

The mass spectrometer measures the mass-to-charge (m/z) ratio of ionized peptides based their behaviour in electromagnetic fields. Fundamentally, a mass spectrometer consists of an ionization source, a mass analyser and a detector[184]. At the ionization source, the peptides of the sample are vaporized and ionized, in our case with electrospray ionization (ESI)[185]. They then enter the mass analyser where electrodes create an electromagnetic field. As the properties of the electromagnetic field are known and carefully controlled, the m/z-ratio for the ionized peptides can be calculated based on how they pass through the instrument and then encounter the detector[186]. In LC-MS/MS, MS is performed twice. In the first round (MS1), peptides, which are referred

to as precursors, are separated based on m/z-ratio. Precursors are then fragmented before the second round of MS (MS2), where the m/z-ratio of the fragments are measured. As precursor peptides with different amino acid sequence may have the same m/z ratio, the second round of MS increases the specificity of the peptide identification[187].

The output from the detectors of the instrument results in a large number of spectrograms with peaks for different m/z-ratios and their respective intensity. By analysis of the spectrograms, the relative content of different peptides and their corresponding proteins can be established, either by targeted methods, such as selected reaction monitoring (SRM) or non-targeted methods such as data dependent acquisition (DDA) or data independent acquisition (DIA). Targeted methods refer to the fact that specific predefined peptides are measured. Conversely, non-targeted methods attempt to quantify as many proteins as possible making them more suitable for studies with the aim of new discoveries or hypotheses, such as Paper IV. In DDA peptides are measured in MS1 and the most abundant ones are selected, fragmented and identified in MS2 through matching of the spectrogram to computer generated theoretical spectra of different peptides. Limitations of this approach are that peptides with low abundance are not measured and that the quantification is less accurate due to it being performed in MS1 where more noise is present in the measurement. In DIA all peptides within a specific m/z-ratio interval are fragmented and measured simultaneously in MS2. This is repeated on different m/z-intervals to cover the entire m/z-range of interest. This approach increases the number of peptides that can be quantified and also increase the resolution of the data[188]. Parallel accumulation–serial fragmentation (PASEF) is a development of MS/MS technology that allows increased throughput in experiments by performing multiple MS/MS scans at high speed without losing sensitivity[189]. At first the method could only be used in DDA-methods, but has later been extended to DIA-methods, referred to as diaPASEF[190], which was used in Paper IV.

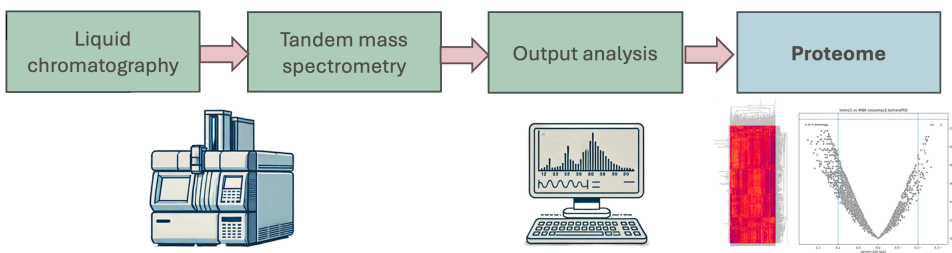

Figure 7: Schematic illustration proteome analysis with LC-MS/MS.

Procedure

Briefly, 40 µL of CSF was pre-processed with the ENRICH-iST kit 96x (PreOmics, Planegg/Martinsried, Germany), according to the manufacturer's instructions. After resuspension, peptide concentration of each sample was determined by NanoDrop. All samples were then diluted similarly based on the median peptide concentration of all samples. Peptide clean-up was performed on disposable Evotip C18 trap columns (Evosep Biosystems, Odense, Denmark) according to the manufacturer's instructions.

A timsTOF HT (Bruker Daltonics, Bremen, Germany) mass spectrometer with a *diaPASEF* method was used for LC-MS/MS. The 30 samples per day (SPD)-protocol was used. In each cycle, the first spectra was acquired in full scan mode (MS1) to record m/z intensities and ion mobility (IM) values for precursor ions, followed by 12 acquisitions of diaPASEF spectra (MS2) to record m/z intensities and IM values for peptide fragment ions. For each diaPASEF scan, there were 2 IM windows to cover the selected IM range.

Data processing and analysis

DIA MS results in spectrograms with peaks for multiple peptides within the same m/z range and significant computational power is required to translate spectrogram data into peptide precursor quantities. In our case, we used DIA-NN (Data-Independent Acquisition by Neural Networks) software[191] and the Uniprot Reviewed proteins (UP000005640 release 2023_02) database to achieve this. At precursor level, false-discovery rate (FDR) was filtered at 1%.

Precursor quantification data was then normalised and log2 transferred before conversion to protein quantification data that could be visualised and statistically analysed. These steps were performed using algorithms in the data processing kitchen sink (DPKS) package[192]. Expression of individual proteins were then compared between controls without CNS infection and EVDRI- and ABM subjects respectively, using student's t-test. Due to the multiple comparisons performed, Benjamini-Hochberg correction[193] was applied to all obtained p-values. Corrected p-values and log2-fold changes between the groups for individual proteins were plotted in volcano plots. Expression of the proteins that could be measured in all samples in the cohort were also visualized in a heat map and stratified according to underlying diagnosis and proteome similarity using PyComplexHeatmap 1.7.6.

Results

Paper I

Participants

Out of 228 consecutive patients with spontaneous SAH treated with an EVD Uppsala University Hospital between January 2010 and December 2013, 37 fulfilled one or several exclusion criteria, leaving 191 subjects for inclusion.

Key findings

A positive CSF culture was obtained in 18 subjects (9.4%). Based on this study's definition, 11 subjects developed EVDRI, resulting in an incidence of 5.8% per patient, 5.4% per EVD and 4.1 per 1000 days of EVD treatment. Subjects with EVDRI had longer duration of EVD treatment, larger volumes of CSF drained, and more CSF samples collected but there were no differences regarding age, gender or clinical or radiological SAH severity.

Out of the 11 cases of EVDRI 9 were caused by CoNS, 1 by *Klebsiella pneumoniae* and 1 by *Staphylococcus aureus*. The CSF culture findings not considered EVDRI were 4 cases of CoNS and one case of *Micrococcus* species, alpha-hemolytic streptococci and diphteroids, respectively.

In 81 of the subjects (42.4%), empirical EVDRI therapy was initiated on 97 occasions during the neuro-ICU care. Based on the CSF cytochemistry findings, the clinical action regarding CSF culture and initiation of EVDRI therapy were in line with the local clinical guidelines in 307 of 592 occasions (51.9%). The guidelines showed poor performance in prognosing a positive CSF culture and only 11 of 22 (50%) positive CSF cultures were from samples that fulfilled cytochemical criteria for performing CSF culture, according to the guidelines.

Paper II

Participants

In total, 103 consecutive patients, that had CSF sampled from an EVD at the neuro-ICU of Skåne University Hospital in Lund between January 2009 and March 2010 were included. Of these, 13 had ABM or ventriculoperitoneal shunt infection as the cause of admission and were not assessed regarding EVDRI, leaving 90 subjects for this assessment.

Key findings

CSF culture or PCR was positive in 16 out of 90 (17.8%) subjects eligible for EVDRI assessment. Based on this study definition of EVDRI, 7 of these subjects had EVDRI (7.8%) and in the remaining 9 subjects (10.0%) with a positive CSF culture or PCR, the findings were considered colonisations or contaminations. In the 7 subjects with EVDRI, CoNS was the aetiology in 5 cases, *Escherichia coli* in 1 case and *Streptococcus oralis* followed by *Candida albicans* in 1 case. The microbiological findings defined as contaminations or colonisations were CoNS in 6 cases, *Micrococcus* species in 2 cases and *Streptococcus mitis* in 1 case.

The concentration of HBP in CSF was higher in EVDRI subjects than in non EVDRI subjects (7.60 ng/mL [IQR 4.1–24.5 ng/mL] vs 31.7 ng/mL [IQR 26.9–40.7 ng/mL], p=0.024) when comparing the HBP at the time of positive culture or PCR in EVDRI subjects to peak HBP of non EVDRI subjects. There was however significant overlap in CSF HBP concentrations between the groups (Figure 8).

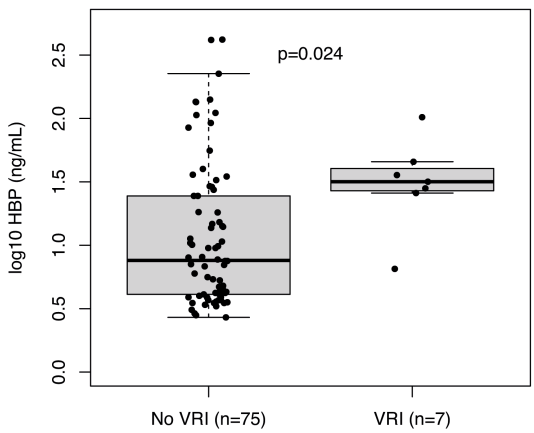

Figure 8: CSF HBP of non-EVDRI vs EVDRI subjects.
HBP concentration at time of EVDRI diagnosis was used for VRI subjects. Peak CSF HBP without EVDRI treatment was used for non-EVDRI subjects. Median HBP was 7.60 ng/mL (IQR 4.1–24.5 ng/mL) for non-EVDRI subjects and 31.7 ng/mL (IQR 26.9–40.7 ng/mL), (p=0.024).

To assess the diagnostic performance of HBP in CSF as a biomarker for EVDRI, the area under the curve (AUC) of a receiver operating characteristic curve (ROC-curve) was calculated (Figure 9) and estimated to 0.76 (95% confidence interval [CI], 0.62-0.90). This corresponded to a sensitivity of 85.7% and a specificity of 75.7% using a cut-off value of 25.15 ng/mL and a negative and positive predictive value of 0.98 and 0.26, respectively, in a setting with a 7.8% prevalence of EVDRI.

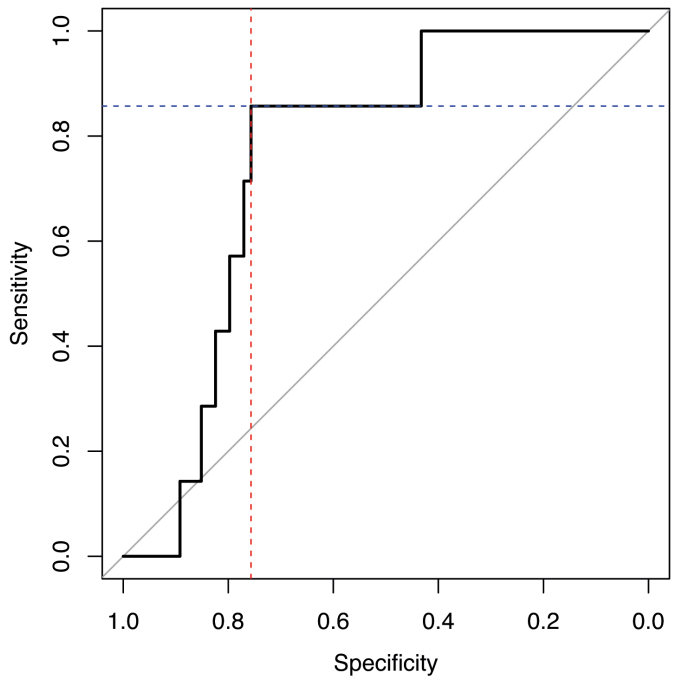

Figure 9: ROC-curve of CSF HBP to diagnose EVDRI.
The red and blue lines indicate the specificity and sensitivity of the optimal cut-off value of HBP (25.15 ng/mL).

The CSF HBP values were also compared between groups of subjects with different causes of admission to the neuro ICU. In this analysis subjects with ABM and SI were also included. Median peak CSF HBP concentrations were highest in subjects with ABM.

Paper III

Participants

Out of 96 prospectively included patients with clinical suspicion of EVDRI at the neuro-ICU or neuro-intermediary care unit between February 2018 and November 2021, 12 were excluded due to one or several exclusion criteria, leaving 84 subjects for analysis. Out of these, 22 had a positive CSF culture, 18 were CSF culture negative with no antimicrobial therapy at sampling and 44 were culture negative with antimicrobial therapy at sampling.

Key findings

Out of the 22 subjects in the culture positive group, 11 had CoNS, 6 had *Staphylococcus aureus,* and there was 1 case each of *Enterococcus faecalis*, *Enterobacter/Klebsiella aerogenes, Escherichia coli*, *Bacillus cereus* and *Micrococcus luteus*.

Real-time 16S PCR was positive in 17 of the 22 subjects in the culture positive group and negative in 61 of the 62 subjects in the culture negative groups. This resulted in a sensitivity and specificity of real-time 16S PCR compared to culture of 77% and 98%, respectively. Based on prescribed antimicrobial therapy, 3 out of the 5 samples with positive culture and negative PCR were clinically regarded as contaminations.

In 16S Nanopore sequencing, the number of 16S sequence reads were higher among culture positive subjects compared to both culture negative groups (99 861 [IQR 19 043–99 982] reads vs. 1174.5 [IQR 288–3 992] and 1300 [IQR 335–3 969]), respectively ($p<0.001$). There was no difference between neither of the culture negative groups and the negative control group. Figure 10 summarize the findings regarding number of Nanopore 16S reads and real-time 16S PCR positivity/negativity.

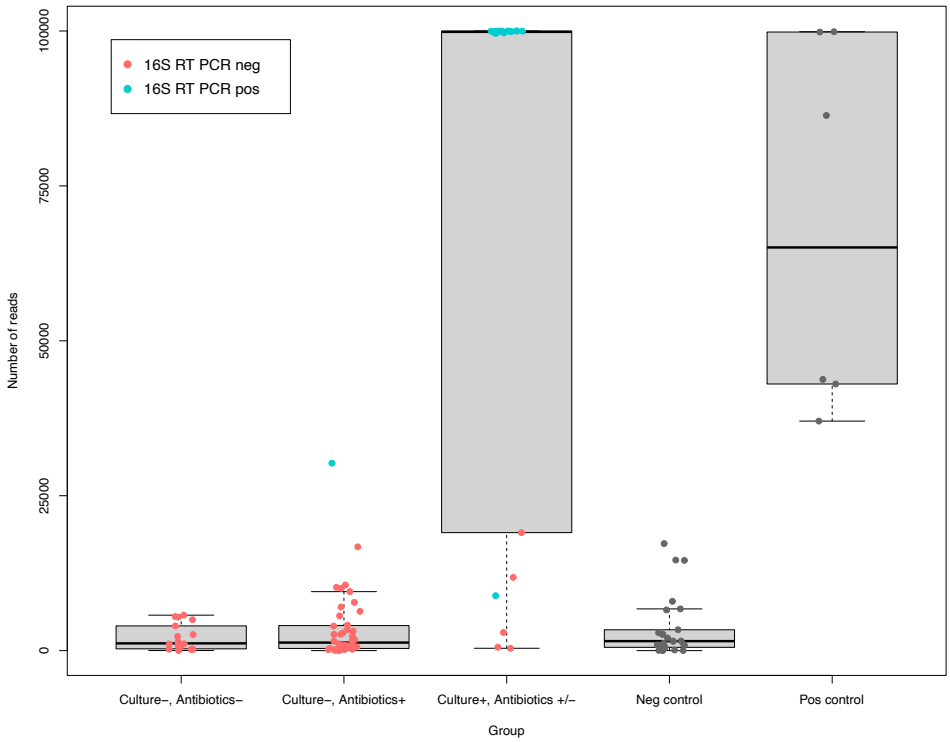

Figure 10: Number of 16S sequence reads in Nanopore sequencing in the study groups. Each dot represent one subject and the color represent 16S real-time positivity (light blue) and negativity (coral). RT-PCR=Real-time PCR.

Species identification with 16S Nanopore sequencing was concordant with the identification of cultured bacteria in 20 of 22 cases. In one case, Staphylococcus aureus was identified in culture and 16S Nanopore sequencing identified *Staphylococcus haemolyticus* and *epidermidis* sequences. In another case, *Micrococcus luteus* was identified in culture and Nanopore sequencing showed low numbers of unclassified sequences and sequences from *Acinetobacter tandoii*.

To investigate if there were signs of false negative CSF cultures, 16S sequencing data was assessed for 17 subjects with 16S sequence reads above the third quartile among culture negative subjects. The subject with the highest number of 16S sequence reads (30 258) was also positive in real-time 16S PCR and the sequence reads were classified to several different bacterial species, where *Variovorax paradoxus* and *Massilia eurypsychrophila* showed the highest relative abundance. Among other culture negative subjects with high read numbers, there were generally high numbers of unclassified reads and reads from different environmental bacteria. Details are shown in Table 8.

Table 8: Details of culture negative samples with high number of 16S reads.
Bacteria with a relative abundance of ≥10% are listed.

Subject	Number of 16S reads	Nanopore species identification	% of reads	Antibiotics at sampling	Real-time 16S PCR (Δ-Cp)
Neg1	10216	Unclassified *Moraxella oblonga*	55.1 30.1	Yes	Neg
Neg2	7035	*Staphylococcus epidermidis/haemolyticus/hominis* *Staphylococcus lugdunensis* *Hungateiclostridium clariflavum*	31.2 30.6 24.1	Yes	Neg
Neg3	7792	Unclassified *Pseudomonas poae* *Pseudomonas bohemica*	56.5 13.8 12.0	Yes	Neg
Neg4	9523	*Pseudomonas stutzeri* *Alcaligenes faecalis*	52.3 47.2	Yes	Neg
Neg5	5610	*Enhydrobacter aerosaccus* *Acinetobacter calcoaceticus* *Acinetobacter pittii*	49.2 22.6 19.0	Yes	Neg
Neg6	16755	*Streptococcus sanguinis* *Delftia acidovorans* *Streptococcus oralis*	24.1 27.1 14.9	Yes	Neg
Neg7	3969	Unclassified	99.1	Yes	Neg
Neg8	4122	Unclassified *Escherichia coli*	49.9 30.1	Yes	Neg
Neg9	10059	*Delftia acidovorans* *Ochrobactrum anthropi*	57.0 32.6	Yes	Neg
Neg10	6342	*Anaerobacterium chartisolvens*	66.1	Yes	Neg
Neg11	10597	Unclassified	84.4	Yes	Neg
Neg12	30258	*Variovorax paradoxus* *Massilia eurypsychrophila*	20.4 18.8	Yes	Pos (3.68)
Neg13	5402	Unclassified *Serratia grimesii* *Serratia plymuthica*	41.3 19.7 19.1	No	Neg
Neg14	4987	Unclassified *Ralstonia pickettii*	39.5 20.9	No	Neg
Neg15	5725	Unclassified	98.7	No	Neg
Neg16	5487	*Delftia acidovorans* *Ochrobactrum anthropi* *Sphingopyxis solisilvae*	56.9 21.1 11.1	No	Neg
Neg17	3992	*Cutibacterium acnes*	96.9	No	Neg

Paper IV

Participants

The same cohort was used as in paper III. However, in paper IV, subjects with ABM were also included for comparison with EVDRI subjects. Out of 97 prospectively included patients with either clinical suspicion of EVDRI or confirmed ABM, 5 were excluded due to one or several exclusion criteria, leaving 92 subjects in the study. Subjects were divided into one of three groups: EVDRI (defined as a positive CSF culture in patient with clinical suspicion of EVDRI), controls with no EVDRI (defined by negative CSF culture) and ABM (defined as clinical presentation of ABM with CSF culture, CSF PCR or blood culture with a relevant ABM pathogen). There were 22, 62 and 8 subjects in the EVDRI, controls and ABM groups, respectively.

Key findings

Although the same study cohort was used as in paper III, due to subtle differences in exclusion criteria the composition of the EVDRI group was slightly altered. One subject with *Staphylococcus aureus* was excluded as no sample within the specified time bins was available, and one case with *Staphylococcus epidermidis* and *Staphylococcus capitis,* not included in paper III as the sample with positive culture was not available for 16S PCR analysis, was included in paper IV. This resulted in 12 cases of CoNS, 5 cases with *Staphylococcus aureus* and one case each of *Enterococcus faecalis, Bacillus cereus, Enterobacter species, Echerichia coli* and *Micrococcus luteus*.

In the ABM group there were 5 subjects with *Streptococcus pneumoniae* and one case each of *Listeria monocytogenes, Enterobacter cloacae* and *Streptococcus anginosus*. The subject with *Listeria monocytogenes* had no positive CSF microbiology but a positive blood culture and CSF pleocytosis and CSF samples were collected during antimicrobial treatment. The other ABM subjects had either a positive CSF culture, PCR or both.

In the comparison of protein expression between EVDRI subjects and controls none of the compared proteins exceeded the thresholds to be regarded as differentially expressed at either of the time bins. When comparing the protein expression of ABM subjects to controls, no proteins reached thresholds for differential expression either. However, a large number of proteins approached a corrected p value of 0.05 and had log2-fold changes of up to almost 4. The results are displayed in Figure 11.

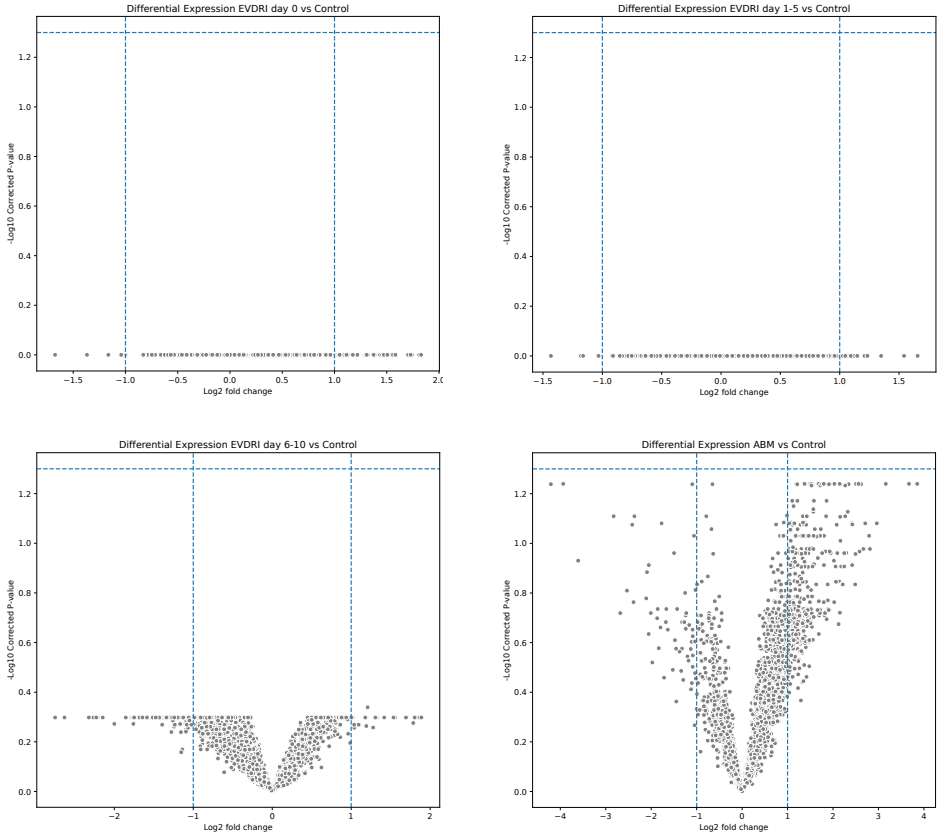

Figure 11. Differential expression of proteins between controls and EVDRI AND ABM subjects. For EVDRI subjects, samples collected at day 0, day 1-5 and day 6-10 were compared to controls. For ABM subjects the first available sample was compared to controls. No proteins met the predefined conditions to be considered differentially expressed (dashed blue lines).

Discussion

EVDRI incidence

Since EVD-devices were first introduced, infections have been a complication of concern. As described in the background chapter, the incidence of these infections varies considerably between studies and sites. The EVDRI incidence of 5.8% per patient and 4.1 per 1000 EVD days observed in paper I is below the mean incidence of 8.8% per patient and 11.4 per 1000 EVD days described in the review and meta-analysis by Lozier et al. and Ramanan et al., respectively[34,35]. However, the incidence of 8.8% in Lozier's review was based solely on positive CSF culture. The incidence of a positive CSF culture in the cohort of paper I was 9.4%, slightly higher than the mean of the studies in the review by Lozier. However, paper I only studied patients with SAH, and as previously described, SAH is associated with a higher risk of EVDRI. In addition, it can be speculated that SAH is associated with longer EVD treatment compared to other causes of neuro-ICU admission, leading to higher incidences of EVDRI per patient due to longer exposure. This could be addressed by comparing the incidence per 1000 EVD days, as done in the meta-analysis of Ramanan et al. Here, the reported mean incidence of EVDRI was estimated to 11.4/1000 EVD days (95% CI 9.3–13.5) overall, including studies with different EVDRI definitions. This is considerably higher than both 4.1 EVDRI/1000 EVD days and 6.7 positive CSF cultures/1000 EVD days reported in paper I. This indicates that the incidence of EVDRI is at the lower side of the range in Swedish neuro-ICU settings.

Studying the incidence of EVDRI was not a primary objective in paper II, but due to the consecutive inclusion of EVD patients it provides additional data regarding this matter. In this cohort, not limited to SAH patients, the incidence of EVDRI and positive CSF culture was 7.8%. and 17.8%, respectively. As slightly different definitions of EVDRI were used, results cannot be directly compared. However, it can be speculated that the management practice of collecting CSF samples routinely twice per week, as during inclusion of subjects in paper II, could contribute to the higher incidences of EVDRI and positive CSF cultures per patient observed in paper II.

The incidence of EVDRI in a Swedish setting has previously been studied by both Lundberg and Sundbärg at Lund university[22,47], but modern studies from Swedish neurosurgical centres are lacking. In Lundberg's works on ICP recording CSF was

cultured routinely once or twice per week in 105 patients and 21 samples from 13 patients (12.4%) were positive. However, based on the bacteria cultured and the lack of clinical symptoms of intracranial infection, it was suspected that most of these results were due to sample contamination or bacteria colonising in the distal parts of the tubing[22].

Sundbärg et al. also studied EVDRI, among other complications of ventricular fluid pressure recording. In a cohort of 540 surviving EVD patients between 1982 and 1986, EVDRI was defined as positive CSF culture and WBC of ≥11/μL with ≥50% PMNC. Positive CSF cultures occurred in 10% of patients, and 5.6% fulfilled WBC criteria for infection. These cases were then divided into suspected or definite, based on presence of symptoms of infection. Using this definition, definite and suspected EVDRI developed in 4.3% and 1.3% of all patients. and in 10.0% and 0.9% in the SAH sub-group[47].

By comparing the results of paper I with the works of Lundberg and Sundbärg, it seems that although equipment and management practices for neurosurgical patients has changed over the decades, positive CSF cultures in EVD patients remain at comparable levels, and the same issues of interpreting their clinical significance remain.

Microbiological aetiology in EVDRI

CoNS was the most common type of bacteria encountered in CSF cultures in all patient cohorts included in this thesis. CoNS was found in 72.2%, 68.8% and 50.0% of subjects with positive CSF culture in paper I, II, and III/IV, respectively. This is in line with the 69.5% reported by Sundbärg et al.[47] and 56% reported by Öhrström et al. in Denmark in the 1980s[51]. The slightly lower proportion of CoNS findings in the material of paper III could be because only patients with clinically suspected EVDRI were included in that study, which favours more virulent pathogens, such as *Staphylococcus aureus,* which accounted for 27.2% of the CSF culture findings in that material. While CoNS is also the most common pathogen in other materials, comprising 38% of positive CSF cultures in pooled data[35], the dominance seems to be more profound in Scandinavian materials. There are also individual studies that show a dominance of gram-negative infections[46,130,131,137]. The general dominance of CoNS suggests that the majority of EVD infections and colonisation originate from the bacterial flora of the skin. Why this is not found universally is unknown, but it might be influenced by local bacterial flora, antibiotic use and EVD insertion- and management protocols that differ between different countries and neurosurgical centres.

The dominance of skin commensals in CSF cultures of EVD patients makes positive culture results harder to interpret compared to cases with gram-negative bacteria or *Staphylococcus aureus*, which are typically significant findings that require antimicrobial treatment. This, along with the fact that infections with CoNS are usually associated with less pleocytosis and other signs of CNS inflammation[194], makes studying EVDRI and EVDRI diagnostics more challenging in a CoNS-dominated setting. Furthermore, this must be considered when interpreting results of EVDRI biomarker studies performed in settings with different spectrums of bacterial aetiology.

Diagnostic performance of HBP in EVDRI

HBP has been shown to have potential as a CSF biomarker for ABM[195–198]. In the first study by Linder et al., it was shown that CSF HBP was significantly higher in subjects with ABM compared to subjects with viral CNS infections, neuroborreliosis and negative controls. There were also significant differences between the study groups regarding CSF WBC, CSF PMNC, CSF glucose and CSF protein but the AUC of the ROC curve was highest for CSF HBP[195]. A subsequent study of Kandil et al. confirmed that CSF HBP had a higher AUC of the ROC-curve than conventional CSF biomarkers for discriminating ABM from viral CNS infections, and it was also showed serum HBP could be used in a similar manner[196]. Another study Ren et al demonstrated the same findings in children[197] while a study of Namiduru et al showed CSF HBP could also help discriminate ABM from tuberculous meningitis[198].

However, in patients with clinically suspected ABM, the need for new biomarkers is not as pressing as in EVDRI, as conventional biomarkers usually work sufficiently well. Furthermore, there are not as many confounding factors regarding symptoms, CSF cytochemistry and microbiology findings, and hence, HBP has not been implemented as a routine CSF biomarker for ABM. The findings of these studies on ABM cannot be directly extrapolated to the neuro-ICU setting, but it was clinically motivated to examine whether CSF HBP could also be used to discriminate EVDRI from aseptic CNS inflammation in NIC patients, which was attempted in paper II. The results indicate that HBP does not have the same performance of separating bacterial infection from other causes of CNS inflammation in the neuro-ICU setting. While CSF HBP concentrations were higher in subjects with confirmed EVDRI, there was a significant overlap of the HBP concentrations between the EVDRI and non-EVDRI subjects. This resulted in a relatively modest AUC of 0.76 and a sensitivity and specificity of 85.7% and 75.7%, respectively, when using a cut-off of 25 ng/mL. These results are hard to put into perspective as no comparisons to other CSF biomarkers were performed, but do not indicate that HBP on its own will solve the diagnostic challenges

regarding EVDRI. It may however have clinical utility when combined with other biomarkers.

The findings of paper II are contrasted by those of Kong et al. in their 2022 study on HBP as a marker of nosocomial meningitis and ventriculitis, including EVDRI[98]. This study showed excellent accuracy of CSF HBP with an AUC of 0.99 and a sensitivity and specificity for all nosocomial meningitis of 97% and 95%, respectively, and 100% and 96% respectively for the diagnosis of EVDRI. There is however one key methodological difference regarding infection definition between these studies that can explain the discrepancy in results. Kong et al. used a definition of infection requiring either a positive CSF culture or one clinical symptom of meningitis together with either CSF cytochemical changes (WBC > 100/μL or protein > 50mg/dL or glucose < 2.5 mmol/L) or a positive CSF gram stain or a positive blood culture. The definitions are compared to those of paper II in Table 8. Only 28 of the 131 patients in the infected group had a positive CSF culture, meaning most subjects were allocated to the infection group based on other criteria. The median CSF WBC of the infected group was 220/μL (IQR 809–6 207/μL) compared to 19/μL (IQR 8–39/μL) in the non-infected group. Furthermore, HBP correlated to WBC with a Spearman *rho* of 0.57 which makes the high HBP concentrations in subjects classified as infected unsurprising, given the difference in WBC levels. Hence, this study is, in a sense, investigating the performance of HBP as a biomarker for elevated CSF WBC rather than for infection. This is addressed in a subgroup analysis where CSF HBP is compared between culture positive and negative subjects. While there is still a significant difference between culture positive and negative groups, with a median CSF HBP of 174 ng/mL (IQR 110-214 ng/mL) and 137 ng/mL (IQR 77-184 mg/mL), the difference is small and the ranges overlap considerably, more resembling the findings of paper II. The somewhat conflicting conclusions of these two studies and the small size of the EVDRI group in paper II motivate further studies of HBP in this setting before concluding whether it has clinical value.

Table 9: **Summary of differences in between paper II and Kong et al "Accuracy of heparin-binding protein for the diagnosis of nosocomial meningitis and ventriculitis".**

	Widén et al	Kong et al
Infection definition	Positive CSF culture or PCR and Erythrocyte corrected CSF WBC >50/μL	1. Positive CSF culture or 2. Temperature >38 ℃ or headache or meningeal signs or focal neurological impairments and CSF WBC >100/μL or CSF protein >50 mg/dL or CSF glucose <2.5 mmol/L or Positive CSF gram stain or Positive blood culture
Number of subjects (infected/non-infected)	7/83	131/150
Number of subjects with positive/negative CSF culture	16/74	28/253
CSF HBP, infection vs no infection (median[IQR])	31.7 ng/mL(4.1–24.5 ng/mL) vs. 7.7 ng/mL(26.9–40.7 ng/mL)	140 ng/mL(87–189 ng/mL) vs. 1.2 ng/mL (0.7–3.5 ng/mL)
CSF HBP sensitivity	86%	97%
CSF HBP specificity	76%	95%
AUC of ROC	0.76	0.99
Optimal HBP cut-off	25 ng/mL	23 ng/mL
CSF WBC/CSF HBP correlation (Spearman's *rho)*	0.65	0.57
CSF WBC of infection vs non-infection (median[IQR])	148 (118–358) vs. 23 (4.8–95.9)	2201 (809—6207) vs. 19 (8–39)

Performance and utility of 16S PCR

PCR for the 16S rRNA gene has been shown to bring added value to other diagnostic methods in infections such as endocarditis and bone and joint infections[175]. However, the studies performed on EVDRI are too small and few and results too diverging to make general recommendations regarding implementation and interpretation of 16S rRNA PCR in this condition. The results from paper III show that real-time 16S PCR on CSF has an excellent specificity of 98% and a lower sensitivity of 77% when compared to bacterial culture. However, in two of the five cases with positive culture

and negative real-time PCR, the Nanopore sequencing showed a very low number of 16S sequence reads, suggesting the culture findings may have been due to contamination during sample collection or analysis which would increase the sensitivity to 86%.

In the most recent and methodologically comparable study of 16S PCR based diagnostics for PNBM by Jang et al.[119] , the sensitivity of 16S PCR was identical with 17 of 22 (77%) culture positive samples being PCR positive. However, there is a large difference regarding specificity. Jang and co-authors observed 23 PCR positive samples from 17 subjects, among the 263 samples from 153 subjects with negative culture results. This could be expressed as a decreased specificity of 91% for the PCR assay with culture as the gold standard. However, based on clinical assessment, all culture negative and PCR positive cases were interpreted as genuine infections, resulting instead in the conclusion that 16S PCR with Nanopore sequencing enhances the detection of PNBM compared to culture. This approach could be discussed as it seems clinicians assessing the cases had information regarding PCR results which may have influenced their assessment of cases, which would result in a circle argument.

Regardless, there is still an important difference between the results of Jang et al. and paper III, where only one case of PCR positivity was seen among culture negative cases. This also contrasts to some previous reports of common occurrence of PCR positivity in culture negative CSF samples[112,113,117]. The study by Druel et al.[112] is from 1996 and PCR methodology has progressed significantly since then, making false positive results less common. However, the fact that none of the negative control samples in the study were PCR positive suggest other explanations. Another fact to consider is that the study was performed on a very selected group of patients with a high clinical suspicion of CNS infection, illustrated by a mean CSF WBC of >1 000/μL in both culture positive and negative cases and it could be argued that bacterial infection would be more likely in these cases. The study by Banks et al.[113] is from 2005 and while the 50% rate of PCR positivity in culture negative samples (43/86 samples) is striking at first, 35 of the samples were collected from patients with a positive culture in a previous sample. Persisting PCR positivity after CSF sterilization during treatment is well known and this circumstance explains most of the "false positivity" of 16S PCR in this study. Furthermore, the study cohort include shunt infections and *Cutibacterium acnes* was the most commonly encountered pathogen, and this bacterium is well known to be challenging to grow in culture[199]. The study by Perdigão et al. included only 43 neurosurgical patients of which 4 had a culture positive infection, which limits the conclusions that can be drawn. Subjects were divided into 4 groups depending on clinical suspicion of CNS infection. PCR positivity was 51% among culture negative subjects and was similar across the probable, possible and unlikely infection groups which question the relevance of PCR positivity in this study[117].

The performance of a PCR is influenced by numerous factors such as DNA extraction protocol, primer selection, thermocycling protocol and method of amplicon detection[200]. Thus, it could be argued that the low rate of PCR positivity among the culture negative groups in paper III may be due to a PCR protocol with a low sensitivity, that only detects bacteria in samples with a high bacterial load, and that culture negative patients could still theoretically have infection. However, the fact that most culture positive subjects were also positive in PCR suggests otherwise. Also, as Nanopore sequencing was performed on all study samples, this data provides further insights. The average number of 16S sequence reads were similar in culture negative subjects and negative controls, which does not support that culture- and/or PCR negative infections were common. Also, the microbiome of the culture negative samples was generally similar to that of negative controls and was dominated by environmental bacteria seldom associated with clinical infections. In many samples, significant proportions of the 16S sequences could not be reliably classified. The cause and implications of this is not known. It can, however, be speculated that those sequences represent noise due to unspecific PCR amplification, not related to bacterial content of the sample or stem from limitations in the laboratory procedures or bioinformatics pipeline.

Nanopore sequencing was also shown to show good concordance to phenotypic and MALDI-TOF-based bacterial identification among culture positive cases where only two cases showed different results between the methods. The performance was not compared to Sanger sequencing in paper III, but the hands-on laboratory effort and time required is significantly less for Nanopore sequencing, which means Nanopore sequencing and other NGS sequencing technologies will probably gradually replace Sanger sequencing at clinical microbiology laboratories. The fact that the results also give more information regarding the bacterial load and composition of polymicrobial infections will also increase the clinical utility of 16S PCR assays, while it will also increase requirements on clinical microbiologists and clinicians regarding interpretation of the results. Implementation of Nanopore sequencing will also bring several questions regarding laboratory workflow based on how turnaround times, accuracy and economic cost are prioritised as the platform is highly customisable regarding hardware and bioinformatic pipeline setup.

Overall, the results presented in paper III support that 16S PCR can be valuable in the diagnosis of EVDRI with a high overall concordance to bacterial culture, which is useful due to the faster turnaround time of PCR. However, positive results will still have to be interpreted along with clinical symptoms and CSF biomarkers to assess their clinical relevance.

CSF proteome in EVDRI

No significant differences regarding individual CSF protein expression were observed when comparing subjects with and without EVDRI in paper IV, where state of the art MS was used to obtain the data. This provides an illustration of why EVDRI diagnosis is challenging in the clinical setting. Concentrations of many CSF proteins are affected by the underlying condition, its severity, timing and other unknown individual factors. This heterogeneity and large inter-individual variations of the CSF proteome makes discovery of changes specifically related to EVDRI challenging, which is also illustrated by the limitations in sensitivity and specificity of current and proposed CSF biomarkers. The potential of MS to measure thousands of proteins simultaneously also means all group-wise comparisons must be corrected for multiplicity, which increase the difference required between groups to reach statistical significance. This, in combination with the apparent heterogeneity of the proteome in neuro-ICU patients limits the potential of MS in identifying individual novel CSF biomarkers for EVDRI.

However, with optimised study design and data processing strategies MS could still provide an important tool in unravelling the CSF response to EVDRI. As is always the case, an increased number of subjects would increase the power of the study and with MS technology and data processing strategies constantly being improved, larger sample sizes in proteomic studies are now realistic. Also, with more homogenous groups regarding underlying condition and perhaps also regarding microbiological aetiology in EVDRI cases, the chances of identifying proteomic changes specifically related to EVDRI could be increased. This would, however, limit the clinical applicability of the results. It is also possible that by artificial intelligence aided methods, patterns of proteomic changes related to EVDRI could be identified even though no differential expression of individual proteins was observed.

Conclusions

- In SAH patients with EVD treatment, EVDRI is often suspected and treated compared to the observed incidence. Use of empiric treatment could be reduced with more specific biomarkers and/or faster microbiological methods.
- Elevated HBP in CSF correlates to EVDRI but it has limitations in both specificity and sensitivity that limit its added value as a clinical biomarker.
- PCR for the 16S rRNA gene on CSF has a high concordance with bacterial culture in suspected EVDRI and can guide clinical decisions regarding EVDRI therapy before culture results are available.
- Nanopore sequencing provides additional information regarding bacterial composition and load in EVDRI and it suggests false negative CSF cultures are uncommon in patients with suspected EVDRI, regardless of antimicrobial treatment at sampling.
- There are large individual variations in the CSF proteome of neuro-ICU patients with EVD, possibly related to underlying diagnosis, disease severity, timing and other factors. These differences limit the possibilities of detecting proteomic changes associated with EVDRI with mass spectrometry.

Future perspectives

For future EVDRI research, it is crucial that a more robust and, at least in research settings, more universally adopted definition of EVDRI is established. This has been achieved for other infections that can be challenging to diagnose, such as catheter-related bloodstream infections[201] and infective endocarditis[202], which has allowed for better estimations of incidences, comparisons between studies, as well as assessments of the effectiveness of preventive strategies. Such definitions of EVDRI should be based on microbiological criteria, i.e. positive CSF culture or PCR, in combination with clinical signs of infection for definite diagnosis. More liberal criteria could be used to classify possible infections, as in infective endocarditis definitions[202]. While such criteria will always have to balance requirements of sensitivity and specificity, and their exact composition would have to be thoroughly discussed in expert groups, it is important that such a process is initiated to increase the quality and comparability of all EVDRI research.

Since Nils Lundberg's pioneering works in the 1960s[21,22], continuous measuring of the intracranial pressure has been a cornerstone of neuro-intensive care (NIC). Improvements in technology and surgical techniques have enabled the development of smaller and less invasive devices for intracranial pressure monitoring. However, due to its ability to drain CSF, the EVD will most likely remain a critical tool in the management of NIC patients. Consequently, EVD-related infections will continue to be a reality in neuro-ICUs around the world. While preventing all cases of EVDRI would be impossible, reducing the incidence should be a priority for both clinicians and researchers in the field. Although there is a trend of reduced incidence of EVDRI over time[35], it remains at around 10% or more in several modern studies[62,63,85,174]. Comparing Sundberg's studies of EVD complications from the 1980s[47] with the findings of papers I and II in this thesis, it appears that the situation regarding EVDRIs in Sweden remains quite similar, after more than three decades.

Reduced incidence of EVDRIs can be achieved in multiple ways, beginning with the routines regarding their placement. Even though the evidence is inconsistent regarding the relationship between EVD placement venue and infection risk[203,204], placement in the operating room should provide optimal conditions, minimizing the risk of bacterial contamination, and should be recommended. While it seems that many centres have abandoned the practice of continuous antibiotic prophylaxis during EVD treatment,

perioperative antibiotic prophylaxis remains widespread[27]. The potential benefit of perioperative antibiotic prophylaxis and optimal regimen has not been sufficiently studied and is something that should be further investigated. Optimally, randomised controlled trials would be conducted, but more realistically, intervention studies can be performed as centres modify their routines regarding antibiotic prophylaxis.

As most EVDRI do not develop in the immediate post-operative phase, but usually after several days or even weeks[38,39,75,131], it seems reasonable to assume that many cases of EVDRI are not caused by bacteria inoculated during the EVD placement but rather from later colonization of the EVD catheter. This means optimizing EVD-management routines while the EVD is in place is probably a key factor in reducing the risk of EVDRI. Aside from CSF sampling strategies and interpretation of CSF cytochemistry results, most of the daily EVD management routines are considered nursing tasks. These include draping of the EVD insertion site, hygiene routines of EVD patients and attending staff, and practical management of the CSF collection system and pressure monitor. Optimisation of these factors can potentially reduce EVDRI incidence substantially, as shown in several studies[130,152,153]. A problem with these studies is that they assess the effect of implementing several measures simultaneously as a bundle, often including changes in EVD placement routines and antibiotic prophylaxis regimens. While this approach is practical and increases the likelihood of achieving significant results, it makes it difficult to determine which individual measures are effective. Ideally, future studies should aim to investigate isolated changes in management practices, though this will likely require larger cohorts.

The focus of this thesis has been diagnosis of EVDRI using biomarkers and microbiological methods. The methods primarily in use today, i.e. CSF cytochemistry (WBC, lactate, protein, albumin) and bacterial culture are insufficient to reliably predict and/or diagnose EVDRI. This is illustrated by the thigh rate of empirical EVDRI treatment in paper I and the high rate of positive CSF cultures with uncertain relevance in paper II. Regarding novel biomarkers, HBP does not seem like a solution to the problem of unspecific CSF biomarkers, but it might be of value as a complement to current CSF biomarkers. This must, however, be assessed in larger studies. Paper IV has demonstrated the difficulty of finding specific CSF biomarkers for EVDRI and highlighted the heterogeneity of the CSF proteome in patients with EVD. There will, however, be more attempts of discovering and assessing CSF biomarkers for EVDRI. Given the relative infrequency of the condition and the limited number of patients in most neuro-ICUs, these studies would benefit from being multi-centre to reach sufficient power over a reasonable time.

Bacterial culture of CSF has been the dominating microbiological method used to diagnose EVDRI. Although laboratory methods for bacterial culture have been continuously refined, and species identification of findings has been revolutionised with the implementation of MALDI-TOF technology, some limitations remain. Firstly, the

relatively long time from sampling to results, secondly the difficulties in culturing some bacteria, and thirdly the risk of false negative results due to antibiotic treatment. Bacterial culture will remain a cornerstone in clinical microbiology, but it will to a greater extent be complemented with PCR methods. Paper III demonstrated that both real-time PCR and Nanopore sequencing of the 16S rRNA gene are useful in the diagnosis of EVDRI and could be used to guide decisions regarding EVDRI treatment. Further studies should prospectively investigate whether EVDRI treatment decisions can safely be based on PCR results while awaiting confirmation from bacterial cultures. If this can be demonstrated in EVDRI and other infections, there is rationale to optimize laboratory workflow to allow for shortened times from sampling to PCR results. With Nanopore sequencing this could theoretically be achieved within hours, with real-time sequencing results[205]. This would mean that the time from sampling to results would be nearly the same as for conventional CSF biomarkers. In turn, this could help reduce the issues caused by non-specific biomarkers.

Significant efforts have been made to discover and evaluate sensitive and specific biomarkers for EVDRI, but in clinical practice, little has changed over the past few decades. The apparent heterogeneity of the CSF proteome in patients with EVD, as observed in paper IV, along with the mixed results in assessing various CSF biomarkers, raises the question of whether the discovery and evaluation of CSF biomarkers related to the host response to infection should be a primary focus of future research. Given the increased power and availability of modern microbiological methods, one could argue that researchers and clinicians should instead focus more on diagnostic strategies that directly target the bacteria causing the infection, rather than the host response.

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About the author

Johan Widén works as an infectious disease specialist at Skåne university hospital in Lund and has a special interest in central nervous system infections. In his thesis different aspects of diagnosing central nervous system infections related to external ventricular drains in patients at the neurosurgical intensive care unit are examined.

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