Aus der Kinderklinik und Kinderpoliklinik
im Dr. von Haunerschen Kinderspital
Klinik der Universität München

Direktor: Professor Dr. Dr. Christoph Klein

Implementation of a multiplex PCR Meningitis/Encephalitis Panel as routine diagnostic in an academic tertiary care pediatric hospital

Dissertation
zum Erwerb des Doktorgrades der Medizin
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von

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aus

München

Jahr

2023

Mit Genehmigung der Medizinischen Fakultät
der Universität München

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Tag der mündlichen Prüfung:	16.02.2023

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Table of Contents

Abbreviations

-	CMV	cytomegalovirus
-	CNS	central nervous system
-	CSF	cerebrospinal fluid
-	DoT	Days of Therapy / Therapietage
-	*E. coli*	*Escherichia coli*
-	FA ME Panel	FilmArray Meningitis/Encephalitis Panel / FilmArray Meningitis/Enzephalitis Panel
-	GBS	group B *Streptococcus*
-	*H. influenzae*	*Haemophilus influenzae*
-	HHV-6	human herpesvirus 6 / humanes Herpesvirus 6
-	HHV-7	human herpesvirus 7
-	HSV	herpes simplex virus / Herpes-simplex-Virus
-	*L. monocytogenes*	*Listeria monocytogenes*
-	LoT	Length of Therapy / Therapielänge
-	LP	lumbar puncture / Lumbalpunktion
-	mPCR	multiplex polymerase chain reaction / Multiplex-Polymerase-Kettenreaktion
-	*N. meningitidis*	*Neisseria meningitidis*
-	PCR	polymerase chain reaction / Polymerase-Kettenreaktion
-	PIVC	peripheral intravenous catheter
-	*S. pneumoniae*	*Streptococcus pneumoniae*
-	VZV	varicella-zoster virus
-	ZNS	Zentralnervensystem

List of publications

Publication I

Eichinger A, **Hagen A**, Meyer-Bühn M, Huebner J. Clinical benefits of introducing real-time multiplex PCR for cerebrospinal fluid as routine diagnostic at a tertiary care pediatric center. Infection 2019;47(1):51-8.

Publication II

Hagen A, Eichinger A, Meyer-Buehn M, Schober T, Huebner J. Comparison of antibiotic and acyclovir usage before and after the implementation of an on-site FilmArray meningitis/encephalitis panel in an academic tertiary pediatric hospital: a retrospective observational study. BMC Pediatr. 2020;20(1):56.

1 Introduction

1.1 Important infections of the central nervous system: meningitis, encephalitis, meningoencephalitis

Infections of the central nervous system (CNS) can involve different parts of the brain. Meningitis refers to an inflammation of the meninges and encephalitis to an inflammation of the brain parenchyma. Often the inflammatory process affects both, which is then defined as meningoencephalitis.[1] Meningitis and encephalitis can be life-threatening diseases and have usually a high morbidity and mortality [2–6]. They can be caused by several infectious agents [7,8] and require early diagnosis and treatment [2,9]. Non-infectious causes are an important differential diagnosis [10–12], however, these will not be discussed in this thesis.

1.1.1 Epidemiology and causative pathogens

Meningitis and encephalitis can be caused by several different pathogens, including viruses, bacteria, and on rare occasions fungi and parasites [7,8,11,13,14]. Depending on the patient's age, immune status and the epidemiology of the infectious agent, different pathogens are more likely to be detected [15]. However, in many cases the etiology remains unknown [6,11–13,16,17]. Within the pediatric population, the youngest are most at risk for CNS infections [5,6,13,18–20]. Fungi, such as *Cryptococcus neoformans*, and parasites, such as *Toxoplasma gondii*, are rare, particularly in children, and mainly affect immunocompromised patients [8,11,13,14]. In the following we will further discuss epidemiology, etiology, symptoms, diagnostic and therapeutic procedures of acute bacterial and viral meningitis and encephalitis.

Both, meningitis and encephalitis are mostly caused by viruses [8,11,13,21]. There is a wide variety of viruses causing meningitis and encephalitis, such as enteroviruses, parechoviruses, arboviruses, respiratory viruses (e.g. adenovirus, influenza virus), mumps, measles, rubella, and herpesviruses (herpes simplex virus (HSV) type 1 and 2,

varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus, human herpesvirus 6 (HHV-6) and 7 (HHV-7)) [8,21,22]. Herpesviruses can establish latency and can cause disease either by primary infection or by reactivation [10].

In patients with viral meningitis, enteroviruses are by far the most commonly identified pathogens in all age groups [16,17,21,23]. In temperate climates, most enterovirus infections occur in summer and autumn [17,21]. HSV is among the most frequent pathogens causing sporadic encephalitis globally [8,22,24], occurring in all seasons [22].

In infants and children, important bacterial agents causing acute bacterial meningitis are group B *Streptococcus* (GBS*), Escherichia coli (E. coli), Listeria monocytogenes (L. monocytogenes), Haemophilus influenzae (H. influenzae)* type b, *Streptococcus pneumoniae (S. pneumoniae), and Neisseria meningitidis (N. meningitidis)* [25]. GBS and *E. coli* are the most common bacterial pathogens in neonates [26–28], and *S. pneumoniae* and *N. meningitidis* in adults and children beyond the neonatal period [18,26,29–33].

The incidence of meningitis is higher than that of encephalitis [2,16]. Viral meningitis, with an annual incidence of 20/100,000 inhabitants, is much more common than bacterial meningitis [34]. Viral meningitis usually has a good prognosis [17,23]. While bacterial meningitis and HSV encephalitis are rather rare diseases in Western countries [35–37], these are associated with significant long-term morbidity and mortality [3–5,24].

1.1.2 Clinical presentation

The clinical presentation of meningitis and encephalitis varies with the patient's age, immune status, the causative pathogen, the duration of illness and the anatomic region of the brain that is affected [1,8,25,38,39]. Meningitis typically presents with systemic symptoms (e.g. fever, headache) and signs of meningeal irritation (e.g. nuchal rigidity) [40,41], while encephalitis is usually associated with symptoms resulting from brain parenchymal involvement, such as seizures, altered mental status, or focal neurologic findings [9,42].

In pediatric patients symptoms can be non-specific, subtle, variable or missing [8,25,38] and no specific symptom or sign is pathognomonic [2,38,43]. The clinical manifestations of infectious and noninfectious CNS diseases [22,38,44], and those of meningitis and encephalitis [8,22] may overlap. In addition, some symptoms that may be suggestive of a certain pathogen, e.g. petechiae and purpura for meningococcal disease, may also be seen with other pathogens [38]. The clinical presentation of viral meningitis may be milder than that of bacterial infection [45], though, it is difficult to differentiate between

the different types of infection (e.g. viral vs. bacterial) using clinical information only [23,44,46]. Moreover, the younger a child the less specific the clinical manifestations and the less frequently signs of meningeal irritation are seen [7,19,39]. Young infants often present with non-specific symptoms, such as fever, respiratory distress, poor feeding, vomiting, diarrhea, lethargy, or jaundice [38,47]. Hence, in young febrile infants undergoing evaluation for sepsis, a lumbar puncture (LP) is regularly performed [48,49].

1.1.3 Traditional cerebrospinal fluid examination methods

The diagnosis of meningitis and encephalitis depends on cerebrospinal fluid (CSF) examination [15]. There are several different traditional diagnostic methods that can be performed, including CSF profile analysis, culture, microscopic examination, and more targeted tests, such as antigen tests, serological tests of CSF, and polymerase chain reaction (PCR) [50]. The choice of the appropriate test depends on the suspected pathogen [50]. Patient history, physical examination, additional diagnostic tests of other specimens (e.g. blood, such as serum inflammatory markers and culture, nasopharynx, stool, etc.), cranial imaging, electroencephalogram, and clinical prediction models (e.g. bacterial meningitis score) contribute to establish the diagnosis [22,44,51].

Routine CSF examination in case of suspected CNS infection includes CSF profile analysis, Gram stain and bacterial culture [49,50]. PCR has been established as the gold standard for the detection of many viral agents [1,8]. Though, for some viruses, CSF serological testing may be more suitable (e.g. most arboviruses) or a useful complementary test to PCR (e.g. VZV) [42]. In general, the sensitivity of the different diagnostic tests varies by the causative pathogens and every test has the risk for false positive or false negative results. Making a correct diagnosis early is crucial for optimal patient management.

1.1.3.1.1 CSF profile analysis

CSF profile analysis (CSF cell counts and chemistry) can rapidly show the presence of CNS inflammation [50]. CSF indices, including clarity, cell count, differential, total protein and glucose concentration, may aid in differentiating between the different types of infection (e.g. bacterial, viral, fungal, etc.) [25,40]. In patients with acute bacterial meningitis, CSF examination typically shows a cloudy fluid, consisting of a predominance of polymorphonuclear leucocytes, a highly elevated white blood cell count, raised protein concentration, and low glucose and CSF to blood glucose ratio [7,9,25]. CSF profiles in case of viral meningitis and encephalitis usually show a clear fluid, a predominance of

lymphocytes, only mildly elevated white blood cell count and protein concentration, and normal glucose and CSF to blood glucose ratio [9,22,25,52]. Additional biomarkers, such as CSF lactate, may also aid in differentiating between bacterial and aseptic meningitis [44,53,54]. The presence of red blood cells in CSF is often unspecific and may indicate hemorrhagic HSV encephalitis [22,39] or be due to a traumatic LP [4].

However, CSF parameters may be non-specific and difficult to interpret, as abnormalities may be missing and CSF profiles of bacterial and nonbacterial infection may overlap. For example, in patients with bacterial meningitis a normal cell count may be seen in certain circumstances, particularly during the early stage of disease [7] and CSF abnormalities in neonates with bacterial meningitis might be absent [55]. Viral CNS infections in children, especially in young infants, have also been shown to present without pleocytosis [56–58]. Furthermore, a polymorphonuclear predominance, which is usually typical for bacterial infection, may also be seen in cases of viral infection, especially during the early stage of disease [22,52,59]. In addition, the interpretation of certain CSF parameters may be influenced by a traumatic spinal tap, which occurs more frequently in young children [49], and by antibiotic administration prior to LP [44,53,60].

1.1.3.1.2 CSF Gram stain

Gram stain enables visualization of bacteria in CSF, which can rapidly deliver information and may identify the causative microorganism [40,51,61]. It is a well-validated test [51], though, accurate interpretation is dependent on the observer's skills [61]. The sensitivity is influenced by the concentration of organisms in CSF and depends on the causative species [40,51,61,62]. It has an overall sensitivity of 60–90% [61,63] and the bacteria-specific sensitivity can range from 10 to 93% [51]. Furthermore, the yield of Gram stain may be slightly reduced in patients who received antibiotic drugs prior to LP [51,61]. Gram stain specificity is high with ≥ 97% [61,63].

1.1.3.1.3 CSF culture

CSF culture is the gold standard for the diagnosis of bacterial meningitis and allows for subsequent antimicrobial susceptibility testing [40,51]. In patients with bacterial meningitis without prior antimicrobial therapy, positive culture results are seen in 70–85% [7,61]. Specificity is high (almost 100%) [50]. However, antibiotic therapy prior to spinal

tap may decrease the sensitivity of CSF culture [40,51,60,64]. In children, bacterial culture sterility has been shown to occur within few hours after antibiotic administration [65]. Additionally, bacterial cultures may take up to 72 hours or longer for pathogen identification [40].

In contrast, the usage of CSF viral culture as a routine diagnostic method is not recommended anymore and has been largely replaced by PCR, as this method is faster and has a higher sensitivity [22,52,66].

1.1.3.1.4 PCR

PCR is a molecular method that detects pathogens via amplification of pathogen nucleic acids [22,67]. In contrast to culture methods, PCR tests are more rapid, only need small volumes of liquor, and sensitivity remains high even after some days of anti-infective therapy [67,68]. It has become the standard for the diagnosis of many viral CNS infections [1,8]. Viral PCR tests have shown high specificity and sensitivity [67], e.g. sensitivity of 96% and specificity of 99% for HSV PCR in CSF [69]. However, it has been shown that PCR results for HSV may be false negative, particularly among children and during the first 72 hours of illness [42,70–72]. The sensitivity and specificity of enterovirus PCR have been both estimated to be >95% [67].

PCR testing for bacteria is increasingly being used and can be a useful additional test method to Gram stain and culture [51]. It can detect pathogens at a lower concentration in CSF compared to Gram stain and culture [40] and may be particularly helpful in pretreated patients [25,40,68]. Studies have shown higher sensitivity and specificity of bacterial PCRs compared to culture [73–75], however, it does not inform about antimicrobial susceptibility, hence bacterial culture remains necessary [51].

To date, PCR tests usually target one or occasionally two pathogens [76]. However, the use of singleplex PCRs requires physicians to choose targeted pathogens [50]. Targeted testing includes the risk of missing not considered pathogens [77]. Furthermore, running several different tests may be limited by the total sample volume [50]. Moreover, PCR testing may also take a couple of days, if these need to be sent out to a reference laboratory [78].

1.1.4 Empirical anti-infective therapy

Anti-infective treatment of meningitis and encephalitis depends on the disease-causing pathogen [22,61]. Bacterial meningitis requires prompt intravenous antibiotic therapy and

HSV encephalitis requires prompt intravenous acyclovir therapy [22,61,79]. Antiviral treatment recommendations for other viruses, such as acyclovir for VZV, ganciclovir and foscarnet for CMV, or for HHV-6 in immunocompromised patients, do also exist, though these are based on a lower level of evidence [22,80]. Management of non-HSV viral meningoencephalitis, such as enterovirus or human parechovirus infection, involves primarily supportive care [15,81]. A delay in anti-infective treatment for bacterial meningitis or HSV encephalitis has been shown to be associated with unfavorable patient outcome (e.g. severe sequelae, death) [82–88]. Hence, in patients with suspected bacterial meningitis or suspected encephalitis, empiric intravenous antibiotic or acyclovir therapy must be initiated as soon as possible [22,61,79]. If LP is delayed due to the necessity for cranial imaging (e.g. to exclude increased intracranial pressure), other contraindications (e.g. hemodynamic instability), or the inability to collect liquor, blood should be drawn for culture immediately and empiric anti-infective therapy given without further delay [9,49,61,79]. Since it can be a challenge and time-consuming to make the correct diagnosis in children using only clinical information and traditional liquor examination methods, especially in young infants, [38,39,50,58] empiric anti-infective drugs are usually given early [8,51]. Empiric therapy should be maintained until negative results of diagnostic studies are obtained and as long as there is clinical suspicion for infection [8,9]. If an infectious agent is identified, and if in case of bacterial meningitis susceptibility patterns are known, empiric therapy should be adjusted [22,61,79]. However, as bacterial and HSV infections are rather rare and often no causative agent can be found, this often leads to prolonged and unnecessary anti-infective treatment [13].

1.2 Complications of antibiotic and acyclovir usage

The usage of anti-infective drugs is associated with potential risks and complications that may harm the patient.

Antimicrobial usage is a main driver of antimicrobial resistance [89,90]. Especially over- and misuse of antibiotic drugs have contributed to the emergence of drug-resistant bacteria [89]. In hospitalized pediatric patients, antibiotics are among the most commonly prescribed drugs [91–93], while inappropriate antibiotic usage is regularly observed [94–96]. The decreasing effectiveness of antibiotics poses a worldwide threat to human health [97,98], as once-curable infections are becoming difficult to treat [97,98], also raising

healthcare costs [97–99]. However, antibiotic usage is still increasing worldwide [98,100,101] and so is antibiotic resistance [98]. In Europe and the European Economic Area, an estimated 671,689 cases of infections with antibiotic-resistant bacteria occurred in 2015 accounting for 33,110 attributable deaths [102]. The highest burden of antibiotic-resistant infections was seen among infants [102]. Treatment failure in patients with meningitis due to antibiotic-resistant pathogens have been described [103–107].

Furthermore, the administration of anti-infective therapy can be accompanied by medication errors, catheter-associated complications and drug-related side effects.

Medication errors are a common problem in pediatric inpatients [108,109] and anti-infective drugs are among the most commonly involved medication classes in medication errors [108,110,111]. Medication errors, such as giving the wrong dose or drug, giving it to the wrong patient, or using a wrong route or frequency of administration [112,113], can result in patient harm [110,111,114]. A study by Rishoej et al found that 23.5% of medication errors caused harm in pediatric inpatients [110]. In hospitalized children, especially dosing errors are commonly observed medication errors [108,110,115]. Kneen et al found that the initial acyclovir dose was not correct in 74% of children with suspected viral encephalitis [116]. Underdosage of antibiotics in children that are treated for bacterial meningitis has been described [117]. This poses a severe risk for the patient by increasing the risk for unsuccessful treatment or causing death [117].

Moreover, peripheral intravenous catheterization for the administration of anti-infective therapy carries the risk for catheter-related complications. Peripheral intravenous catheter (PIVC) complications are associated with increased morbidity and can range from simpler ones, including infiltration, accidental dislodgement or occlusion, to more severe ones, such as phlebitis and catheter-related infections [118]. The incidence of PIVC complications and failure in children is high with one third of inserted PIVCs failing prior to therapy completion [118]. In addition, the placement of PIVCs in pediatric patients can pose a challenge. Especially in infants and small children who are also often uncooperative, fragile, small veins and adiposity make this common procedure difficult [119]. This often leads to several frustrating and thus time-consuming attempts, resulting in pain and distress for patients and anxiety in parents [119,120]. Negative venipuncture experiences in childhood may also have longer lasting consequences, such as the development of needle phobia, which again may result in further health problems due to the associated avoidance of medical care [120,121].

Furthermore, antibiotic and virostatic usage can lead to short- and long-term side effects. Most antimicrobial- associated adverse events are mild and rapidly reversible on drug discontinuation, however in some cases they can cause severe clinical conditions [122]. There are numerous possible antibiotic-associated adverse effects, including gastrointestinal side effects (e.g. diarrhea), nephrotoxic and hepatotoxic side effects, neurologic side effects (e.g. seizures, ototoxicity), and hypersensitivity reactions (e.g. drug rash, anaphylactic reactions) [122]. Moreover, antibiotic-induced microbial dysbiosis of the child's gut, especially during infancy, may also lead to the development of specific diseases later in life, such as obesity, asthma and neurodevelopmental disorders [123–127]. Intravenous acyclovir therapy can also cause important complications, such as renal impairment [128].

1.3 Introduction of the FilmArray Meningitis/Encephalitis Panel

Novel molecular tests, such as multiplex PCRs (mPCRs), are increasingly being used in clinical practice for several infectious diseases and may facilitate the diagnosis of CNS infections and improve patient care [77,129].

In October 2015, the first mPCR panel, i.e. FilmArray Meningitis/Encephalitis Panel (FA ME Panel), was cleared by the Food and Drug Administration for the simultaneous identification of pathogens causing meningitis and/or encephalitis [130]. The FA ME Panel (bioMérieux) is a fully automated, qualitative, *in vitro* mPCR assay that can aid in the diagnosis of meningitis and encephalitis [131]. It allows for simultaneous testing for multiple pathogens in CSF obtained via spinal tap [131]. It detects 14 common pathogens (6 bacteria, 7 viruses, 1 yeast) of meningitis and/or encephalitis in a single liquor sample: *E. coli* K1, *H. influenzae*, *L. monocytogenes*, *N. meningitidis* (encapsulated), *Streptococcus agalactiae* (GBS), *S. pneumoniae*, CMV, enterovirus, HSV types 1 and 2, HHV-6, human parechovirus, VZV, and *Cryptococcus neoformans/gattii* [131]. A recent systematic review and meta-analysis evaluated the diagnostic test accuracy of the FA ME Panel, which was found to be high [78]. The overall mean sensitivity and specificity were 90% (95% CI 86–93%) and 97% (95% CI 94–99%) and the positive and negative predictive value were 85.1% and 98.7%, respectively [78]. As with every other diagnostic test there is the risk for false positive and false negative results, which were found to be 4% and 1.5% after adjudication, respectively [78]. As with other tests, sensitivity and specificity may

differ by pathogen. Significant areas of concern were described to be false negative results for HSV, false positive specimens for GBS and *S. pneumoniae* as well as the interpretation of positive latent HSV, HHV-6 and CMV infections [78]. Additionally, the panel is a relatively expensive test with a high acquisition cost for the device and high cost for the individual test panel [132]. However, it provides several advantages compared to traditional testing methods. The FA ME Panel is easy to operate and enables healthcare centers which previously could not provide in-house molecular testing to introduce this panel in their on-site laboratory [77]. Furthermore, the FA ME Panel requires only a small sample volume (200µl) for the simultaneous detection of 14 pathogens [131]. This is of advantage, as the CSF specimen volume obtained, particularly in children, may be too low to perform several pathogen-specific tests [133]. In addition, it facilitates pathogen identification by simultaneously testing for a broad range of possible pathogens, some of which might not routinely be tested for and hence go undetected [77,134,135]. Establishing a microbial diagnosis has the potential to optimize the management of pediatric patients, including a reduction of unnecessary anti-infective therapy [56,136,137]. This is likely to be facilitated by the increased diagnostic yield using a multiplex approach. Furthermore, it is a rapid diagnostic test with limited hands-on time and an assay duration of about one hour [131]. This is in sharp contrast to the relatively slow turnaround time of culture methods [40,67,138]. More rapid detection or exclusion of CNS pathogens in children using molecular methods has been shown to be associated with clinical benefits, including reduced length of hospital stay, reduced unnecessary anti-infective therapy, and reduced associated healthcare costs [139–141].

2 Aim of the thesis

In June 2016, an on-site FA ME Panel was introduced into the clinical routine procedures of the Dr. von Hauner Children's Hospital, Munich, for the evaluation of children with suspected meningitis and/or encephalitis. mPCR testing was done in the in-house bacteriological laboratory during opening hours (weekdays 8 am – 4 pm and on weekends 10 am – 12 pm). Before its implementation, viral PCR testing was performed in the central virology laboratory off-site (without weekend routine working hours), which normally took between two and five days to get results. There, singleplex PCR testing was available for most viral pathogens. Children with suspected CNS infection were routinely tested for HSV-1/2 and sometimes for other viral agents. However, clinical practice was at the discretion of the physician, as no specific guidelines or restrictions regarding the selection and extent of microbiological testing existed.[142,143]

The overall aim of this thesis was to assess the consequences of the introduction of the on-site FA ME Panel on diagnostics and therapy and to find the most effective way to use this panel in the routine patient management. In a first study [142], a retrospective analysis of FA ME Panel tests that were done in children with suspected meningitis and/or encephalitis or sepsis-like illness during the first year after its implementation was performed, including a subgroup analysis of patients 8–84 days of age. It summarized the experience after one year of usage of the panel (publication I). In a second study [143], the effect on empiric anti-infective usage was further investigated in more detail and by using a historical control group (publication II).

2.1 Contribution to publications:

The main research work for the first publication was carried out by Dr. med. Anna Eichinger, the first author of this article. This project was supervised by Prof. Dr. med. Johannes Hübner. I took part in the acquisition of the data and in writing of the manuscript.

The main research work for the second publication was carried out mostly by myself. This project was supervised by Prof. Dr. med. Johannes Hübner. I participated in the design of the study, I collected and analyzed the data. Writing of the first draft, including

the creation of tables and figures were done by me. Editing and finalizing of the publication were done by me with the help of Prof. Dr. med. Johannes Hübner, Dr. med. Anna Eichinger, Dr. med. Tilmann Schober and Dr. rer. nat. Melanie Meyer-Bühn. I was responsible for the submission and publication process.

3 Summary in English and German

3.1 Summary

Meningitis and encephalitis can be life-threatening diseases [2] and be caused by numerous infectious pathogens [7,8]. Bacterial meningitis and herpes simplex virus (HSV) encephalitis require immediate anti-infective treatment [22,61,79], while viral meningoencephalitis not caused by HSV is treated primarily by supportive care [15]. Establishing the correct diagnosis in pediatric patients using clinical data and traditional cerebrospinal fluid (CSF) examination methods can be challenging as well as time-consuming [38,39,50]. Hence, due to the possible catastrophic clinical course, empiric antibiotic and/or acyclovir treatment is usually administered early, particularly in young infants [8,51]. Since bacterial and HSV infections are rather rare diseases, and in many cases no causative pathogen can be identified, this often results in prolonged and unnecessary anti-infective therapy [13] and may result in therapy-associated complications. Timely verification or exclusion of a potential pathogen is crucial to be able to early adjust empiric treatment and ensure optimal patient management. New diagnostic methods, such as multiplex PCR (mPCR) assays, may aid to circumvent some of the limitations associated with traditional laboratory methods and hence improve patient management [77].

In June 2016, the Dr. von Hauner Children's Hospital in Munich implemented an on-site FilmArray Meningitis/Encephalitis Panel (FA ME Panel) into the clinical routine diagnostics for the evaluation of patients with suspected meningitis and/or encephalitis. This mPCR enables the simultaneous detection of 14 common pathogens of meningitis and/or encephalitis (7 viruses, 6 bacteria, 1 yeast) in a single CSF sample within approximately one hour (assay duration) [131]. mPCR testing was performed in the on-site bacteriological laboratory during opening hours (Monday to Friday 8 am – 4 pm and on weekends 10 am – 12 pm). Before its implementation on-site, CSF for viral PCR testing used to be sent out to the central virology laboratory, which was able to perform a variety of singleplex PCRs. Usually, no testing was performed on the weekends and it generally took between two and five days to get results. Children with suspected central nervous system (CNS) infection were routinely tested for HSV-1/2 and occasionally for other viral agents. However, there were no specific guidelines or restrictions regarding the choice and extent

of microbiological testing. Thus, clinical practice was at the discretion of the pediatrician.[142,143]

The objective of this thesis was to evaluate the consequences of the implementation of the on-site FA ME Panel on diagnostics and therapy. Furthermore, it should be assessed how the panel can be used most effectively in the routine patient management. For this purpose, two retrospective studies were performed.

The first study analyzed 187 FA ME Panel tests that were performed during the first year after the implementation in children with suspected meningitis and/or encephalitis or sepsis-like illness and summarized the experience after one year of usage of the mPCR. In addition, a subgroup analysis was done in infants 8–84 days of age. Here, patients with positive viral mPCR results (n= 15) were compared to a group of patients without detection of pathogens (n= 8) to assess differences in demographic (gender, seasonality), clinical (medical history, symptoms, empiric anti-infective therapy, length of hospital stay) and laboratory data.[142]

The second study further evaluated the effect of the mPCR on empiric anti-infective therapy. For this purpose, empiric antibiotic and acyclovir usage were compared between 46 patients that were tested by the FA ME Panel (06/2016 to 02/2017) and 46 matched historical control patients (matched by age and suspected CNS infection) of the years prior to the implementation of the mPCR. In this study, all children with suspected meningitis and/or encephalitis who were prescribed empiric antibiotics and/or acyclovir were included; the following exclusion criteria were defined: early onset sepsis within the first week of life (days 0–6), suspected ventricular shunt infection, immunocompromising disease (immunodeficiency, malignancy), proven bacterial meningitis, as well as patients who were transferred to the Dr. von Hauner Children's Hospital with extern liquor analysis already performed, and patients whose empiric therapy was adapted due to the identification of another cause for the presenting symptoms in the initial work-up (e.g. urinary tract infection). One patient in the mPCR group with subdural hematoma and suspicion of abusive head trauma was also excluded. In each study group, 63.0% (29/46) of the patients were infants, of which most were younger than three months of age (79.3% in the control and 82.8% in the mPCR group). Antibiotic and acyclovir usage were analyzed in more detail using two different parameters (Length of Therapy (LoT) and Days of Therapy (DoT)). Further differences between the study groups regarding diagnostics,

such as the number of PCR tests ordered or the number of detected pathogens, and in length of hospital stay were also assessed.[143]

About half (49.2%) of the 187 analyzed spinal taps that were performed during the first year after implementation, were performed in infants. About 80% of all tested infants were younger than three months old. Most patients had no pre-existing illnesses (77% of infants and 69% of children beyond infancy). The percentage of positive mPCR results was relatively low (14.4%). This may be in part due to the fact that the use of the FA ME Panel did not need approval by the Division of Pediatric Infectious Diseases, hence its usage was at the physician's discretion. While infants mainly got a lumbar puncture (LP) due to fever without focus (53%) or suspected early onset sepsis (29%), there was a variety of symptoms in older children leading to LP, a common one being an isolated facial nerve palsy. In areas of endemic lyme disease, the most common verified infectious cause of this symptom in children is Borrelia burgdorferi [144–146], a bacteria that is not included in the panel. As expected, these children had negative mPCR results.

During the first year of mPCR usage, the majority (88.9%) of detected pathogens were viruses (12 enterovirus, 10 HHV-6 and 2 human parechovirus positive mPCR results), the remaining 11.1% bacteria (2 *S. pneumoniae* and 1 *E. coli* K1 positive mPCR result; all confirmed by bacterial culture); no fungus was detected. However, four of the positive HHV-6 results were judged not to be disease causing. In three of these cases a concurrent bacterial infection outside the CNS was identified. In addition, one child with a clinical diagnosis of group B *Streptococcal* sepsis despite negative blood culture, and one neonate with a diagnosis of invasive CNS listeriosis had negative CSF culture and mPCR results. The highest number of positive FA ME Panel results during the first year of mPCR usage was observed in young infants (8–84 days of age). This age group posed the biggest challenge for the attending physicians, since the majority of these patients presented with fever without focus. Young infants with enteroviral infection showed higher CSF cell counts and protein and exclusively presented in summer and early autumn. However, the subgroup analysis in this age group showed no significant differences regarding gender, medical history, symptoms or laboratory values, that may aid pediatricians to differentiate between infants with and without viral infection.

By implementing the in-house mPCR, results were available faster than prior to its introduction. Furthermore, as seen in the second study, after the implementation of the FA ME Panel the number of patients with detected viral agents in CSF was significantly higher

compared to the historical control group (9 with enteroviruses and 5 with HHV-6 vs. 5 with enteroviruses), highlighting that targeted testing includes the risk of missing viral pathogens. In both study groups, most viruses were isolated in infants. Of these infants with a positive viral result, all but one per study group were younger than three months of age. In the control group, the most frequently performed viral PCR tests were HSV PCRs (93.5%) and enterovirus PCRs (39.1%). PCR testing for other viral agents were rather rarely performed and only enteroviruses were detected.

As observed in both studies, rapid verification of viral agents, as well as the early exclusion of HSV in CSF by the mPCR – together with supporting clinical and laboratory data – enabled physicians to avoid empiric antibiotic and/or acyclovir treatment in individual cases. In the subgroup analysis of the first study, a significant reduction in the duration of antibiotic therapy in infants 8–84 days of age with proven viral infection was already shown. In the second study, antibiotic and acyclovir usage (LoT and DoT) were significantly lower in the mPCR group compared to the historical control group. A stratification by age (infants vs. older children) showed that in infants both, antibiotic and acyclovir usage were significantly reduced, while in older children this was only the case for acyclovir treatment. However, in both, the subgroup analysis of the first study, and in the comparison with the historical control group of the second study, no significant differences in length of hospital stay were observed.

To summarize, the implementation of the on-site FA ME Panel allowed for more rapid pathogen detection or exclusion and for simultaneous testing for a larger variety of pathogens on a regular basis. After its introduction, a significantly increased number of viral agents were detected and viruses that were previously only rarely tested for were identified, thus raising awareness for the broader spectrum of possible viral causes of meningitis and encephalitis. Our results suggest that the introduction of an on-site CSF mPCR into a pediatric clinic can lead to earlier optimization of empiric anti-infective therapy, reducing unnecessary antibiotic and acyclovir therapy or even avoiding empiric treatment. However, no significant reduction in length of hospital stay was seen. According to the studies, the age group that benefitted the most from rapid molecular testing were the group of young infants. The FA ME Panel is a relatively expensive diagnostic method [132]. Other strategies using targeted on-site singleplex PCRs (e.g. for HSV, enterovirus

and HHV-6) may be more cost-effective and according to our results potentially sufficient, though their implementation may be more difficult and other infectious agents might be missed.

To use the panel in a more efficient and cost-effective way a more targeted and structured approach than observed in the study is necessary. This may include limiting the more liberal use of the FA ME Panel (i.e. without consulting the pediatric infection team prior to the use of the mPCR) to infants 1–12 weeks of age. Moreover, critical patient evaluation which also takes into account the specific disease course and all other available information (e.g. accompanying symptoms, exposure etc.) can optimize the usage of the panel. The FA ME Panel may be a useful test for young infants with suspected sepsis or meningitis and/or encephalitis and older children with suspected meningitis and/or encephalitis. However, as supported by findings in the study (e.g. false negative mPCR results, clinically irrelevant HHV-6 detections with or without non-CNS bacterial co-infections) the results of the FA ME Panel should be interpreted within the context of the patient (e.g. symptoms) and results from further diagnostic tests, as also recommended by the manufacturer [131]. If suspicion of bacterial or HSV infection remains high despite a negative mPCR result or the detection of another pathogen, empiric anti-infective therapy should be continued and further diagnostic tests performed.

Considering that both studies were performed retrospectively, involved only one single center and had a relatively small sample size with a low percentage of positive test results, further prospective multicenter studies with a larger number of cases are necessary for further evaluation of the consequences of the introduction of an on-site FA ME Panel in a pediatric setting.

In summary, further prospective evaluation of the FA ME Panel and optimization of its usage is warranted. However, the results of the studies already indicate that an on-site FA ME Panel has the potential to be a useful test to early guide clinical decisions and improve patient management, particularly in young infants.

3.2 Zusammenfassung

Meningitis und Enzephalitis können lebensbedrohliche Erkrankungen sein [2] und durch zahlreiche infektiöse Erreger verursacht werden [7,8]. Die bakterielle Meningitis und die

Herpes-simplex-Virus (HSV)-Enzephalitis benötigen eine sofortige antiinfektiöse Behandlung [22,61,79], während die nicht durch HSV verursachte virale Meningoenzephalitis primär supportiv behandelt wird [15]. Die korrekte Diagnosestellung anhand klinischer Daten und traditioneller Liquoruntersuchungsmethoden kann bei Kindern eine Herausforderung darstellen sowie zeitintensiv sein [38,39,50]. Daher wird aufgrund des potentiell katastrophalen klinischen Verlaufs eine empirische antibiotische und/oder Aciclovir-Therapie in der Regel früh verabreicht, vor allem bei jungen Säuglingen [8,51]. Da bakterielle und HSV-Infektionen eher seltene Erkrankungen sind und häufig kein verursachender Erreger identifiziert werden kann, führt dies oft zu einer prolongierten und unnötigen antiinfektiösen Therapie [13], die Therapie-assoziierte Komplikationen zur Folge haben kann. Der zeitnahe Nachweis oder Ausschluss eines potentiellen Erregers ist sehr wichtig, um die empirische Behandlung frühzeitig anpassen und ein optimales Patientenmanagement gewährleisten zu können. Neue diagnostische Methoden, wie Multiplex-PCR (mPCR) Tests, können helfen, einige der Einschränkungen, die mit traditionellen Labormethoden assoziiert sind, zu umgehen und somit das Patientenmanagement zu verbessern [77].

Im Juni 2016 führte das Dr. von Haunersche Kinderspital in München ein FilmArray Meningitis/Enzephalitis Panel (FA ME Panel) als patientennahe Labordiagnostik (POCT) in die klinische Routinediagnostik für die Evaluation von Patienten mit Verdacht auf Meningitis und/oder Enzephalitis ein. Diese mPCR ermöglicht den simultanen Nachweis von 14 häufigen Erregern der Meningitis und/oder Enzephalitis (7 Viren, 6 Bakterien, 1 Hefe) in einer einzigen Liquorprobe innerhalb etwa einer Stunde (Testdauer) [131]. mPCR Tests wurden in dem hauseigenen bakteriologischen Labor während der Öffnungszeiten (Montag bis Freitag 8:00 – 16:00 Uhr und wochenends 10:00 – 12:00 Uhr) durchgeführt. Vor dessen Implementierung vor Ort, wurde Liquor für virale PCR Tests an das Zentrallabor für Virologie verschickt, welches eine Vielzahl an Singleplex-PCRs durchführen konnte. Üblicherweise wurden wochenends keine Tests durchgeführt und es dauerte in der Regel zwischen zwei und fünf Tagen, bis Ergebnisse vorlagen. Kinder mit Verdacht auf eine Infektion des Zentralnervensystems (ZNS) wurden üblicherweise auf HSV-1/2 getestet und fallweise auf andere virale Erreger. Es gab jedoch keine spezifischen Richtlinien oder Beschränkungen hinsichtlich Auswahl und Umfang mikrobiologischer Testungen; d.h. die klinische Vorgehensweise stand im Ermessen des Kinderarztes.[142,143]

Das Ziel dieser Dissertation war es, die Auswirkungen der Einführung des FA ME Panels als POCT auf Diagnostik und Therapie zu erfassen. Zudem sollte evaluiert werden, wie das Panel in dem routinemäßigen Patientenmanagement am effektivsten eingesetzt werden kann. Hierfür wurden zwei retrospektive Studien durchgeführt.

In der ersten Studie wurden 187 FA ME Panel Tests, die innerhalb des ersten Jahres nach Einführung bei Kindern mit Verdacht auf Meningitis und/oder Enzephalitis oder sepsisartigem Krankheitsbild durchgeführt wurden, analysiert und die Erfahrungen nach einem Jahr Verwendung der mPCR zusammengefasst. Zudem erfolgte eine Subgruppenanalyse bei Säuglingen im Alter von 8–84 Tagen, bei der Patienten mit positivem viralen mPCR Ergebnis (n=15) mit einer Patientengruppe ohne Erregernachweis (n=8) verglichen wurden, um Unterschiede in demographischen (Geschlecht, Saisonalität), klinischen (Anamnese, Symptomatik, empirische antiinfektiöse Therapie, Krankenhausaufenthaltsdauer) und laborchemischen Parametern zu erfassen.[142]

In der zweiten Studie wurde der Effekt der mPCR auf die empirische antiinfektiöse Therapie näher untersucht. Hierfür wurde der empirische Antibiotika- und Aciclovirverbrauch zwischen 46 Patienten, die eine FA ME Panel Untersuchung erhalten haben (06/2016 bis 02/2017), und 46 gematchten Patienten einer historischen Kontrollgruppe (gematcht nach Alter und Verdachtsdiagnose) aus den Jahren vor der Implementation der mPCR verglichen. In dieser Studie wurden alle Kinder mit Verdacht auf Meningitis und/oder Enzephalitis, denen eine empirische Antibiotika- und/oder Aciclovirtherapie verordnet worden war, eingeschlossen; folgende Ausschlusskriterien wurden festgelegt: Early-Onset Sepsis innerhalb der ersten Lebenswoche (Tage 0–6) , Verdacht auf Shuntinfektion, Immunschwäche (Immundefekt, Malignom), nachgewiesene bakterielle Meningitis, sowie Patienten, die mit bereits extern durchgeführter Liquordiagnostik in das Dr. von Haunersche Kinderspital verlegt wurden, und Patienten, bei denen eine andere Ursache für die vorhandene Symptomatik im Rahmen der Erstdiagnostik festgestellt und somit die empirische Therapie angepasst wurde (z.B. Harnwegsinfekt). Ein Patient in der mPCR Gruppe mit subduralem Hämatom und Verdacht auf Schütteltrauma wurde ebenfalls ausgeschlossen. In jeder Studiengruppe waren 63,0% (29/46) der Patienten Säuglinge, von denen die meisten jünger als drei Monate alt waren (79,3% in der Kontroll- und 82,8% in the mPCR Gruppe). Der Antibiotika- und Aciclovirverbrauch wurde anhand von zwei verschiedenen Parametern detaillierter untersucht (Therapielänge (LoT)

und Therapietage (DoT)). Zudem wurden weitere Unterschiede der untersuchten Studiengruppen hinsichtlich Diagnostik (u.a. Anzahl angeforderter PCR Tests und Anzahl detektierter Erreger) sowie Krankenhausaufenthaltsdauer evaluiert.[143]

Etwa die Hälfte (49,2%) der 187 analysierten Lumbalpunktionen (LP), die innerhalb des ersten Jahres nach Einführung durchgeführt wurden, betraf Säuglinge. Etwa 80% aller getesteten Säuglinge waren jünger als drei Monate. Die meisten Patienten hatten keine Vorerkrankungen (77% der Säuglinge und 69% der Kinder jenseits des Säuglingsalters). Der Prozentsatz der positiven mPCR Ergebnisse war relativ gering (14,4%). Dies könnte zum Teil darauf zurückzuführen sein, dass der Einsatz des FA ME Panels keine Genehmigung der Abteilung für pädiatrische Infektiologie benötigte und somit dessen Verwendung im Ermessen des anordnenden Arztes stand. Während Säuglinge größtenteils wegen Fieber ohne Fokus (53%) oder Verdacht auf Early-Onset-Sepsis (29%) eine LP erhielten, gab es eine Vielfalt an Symptomen bei älteren Kindern, die zur LP führten. Häufig war hier z.B. eine isolierte Fazialisparese. In Gebieten mit endemischer Lyme-Borreliose ist die häufigste verifizierte infektiöse Ursache dieses Symptoms bei Kindern Borrelia burgdorferi [144–146], ein Bakterium, das nicht in dem Panel enthalten ist. Wie zu erwarten, hatten diese Kinder negative mPCR Ergebnisse.

Die Mehrzahl (88,9%) der detektierten Erreger im ersten Nutzungsjahr der mPCR waren Viren (12 Enterovirus-, 10 HHV-6- und 2 humane Parechovirus-positive mPCR Ergebnisse), die verbleibenden 11,1% Bakterien (2 *S. pneumoniae*- sowie 1 *E. coli* K1-positives mPCR Ergebnis; alle durch die bakterielle Kultur bestätigt); es wurde kein Pilz nachgewiesen. Allerdings wurden vier der positiven HHV-6 Ergebnisse als nicht krankheitsursächlich gewertet. Hier wurde in drei Fällen eine gleichzeitig vorhandene bakterielle Infektion außerhalb des ZNS identifiziert. Außerdem hatten ein Kind mit der klinischen Diagnose einer Gruppe B Streptokokken-Sepsis (trotz negativer Blutkultur) sowie ein Neugeborenes mit der Diagnose einer invasiven ZNS-Listeriose negative Liquorkultur- und mPCR Ergebnisse.

Die höchste Anzahl an positiven FA ME Panel Ergebnissen im ersten Nutzungsjahr der mPCR wurde bei jungen Säuglingen (8–84 Tage alt) beobachtet. Diese Altersgruppe stellte die größte Herausforderung für die behandelnden Ärzte dar, da die Mehrzahl dieser Patienten sich mit Fieber ohne Fokus präsentierte. Junge Säuglinge mit Enterovirusinfektion wiesen im Liquor höhere Zellzahlen und Proteingehalt auf und erschienen ausschließlich im Sommer und Frühherbst. Die Subgruppenanalyse in dieser Altersgruppe

zeigte jedoch keine signifikanten Unterschiede bezüglich des Geschlechts, der Anamnese, Symptomatik oder Laborwerte, welche Kinderärzten helfen könnten, zwischen Säuglingen mit und ohne viraler Infektion zu unterscheiden.
Durch die Einführung der mPCR als POCT waren Ergebnisse schneller verfügbar als zuvor. Zudem sah man in der zweiten Studie, dass die Anzahl der Patienten mit im Liquor detektierten viralen Erregern nach der Implementierung des FA ME Panels signifikant höher war als im Vergleich zu der historischen Kontrollgruppe (9 mit Enteroviren und 5 mit HHV-6 vs. 5 mit Enteroviren). Dies zeigt auf, dass zielgerichtetes Testen das Risiko birgt, dass virale Erreger unentdeckt bleiben. In beiden Studiengruppen wurden die meisten Viren bei Säuglingen isoliert. Alle Säuglinge, bei denen ein Virus nachgewiesen wurde, waren – bis auf ein Säugling pro Studiengruppe – jünger als drei Monate. In der Kontrollgruppe waren die meist durchgeführten viralen PCR Tests HSV-PCRs (93,5%) und Enterovirus-PCRs (39,1%). PCR Tests für andere Viren wurden eher selten durchgeführt, und es wurden nur Enteroviren detektiert.
Wie in beiden Studien beobachtet, ermöglichten der schnelle Nachweis viraler Erreger sowie der zeitnahe Ausschluss von HSV im Liquor durch die mPCR es den Ärzten – in Zusammenschau mit weiteren klinischen und laborchemischen Informationen – in einzelnen Fällen, auf eine empirische Antibiotika-und/oder Aciclovirbehandlung zu verzichten. In der Subgruppenanalyse der ersten Studie zeigte sich bereits eine signifikante Reduktion der Antibiotikatherapiedauer bei Säuglingen im Alter von 8–84 Tagen mit viralem Erregernachweis. In der zweiten Studie war der Antibiotika- und Aciclovirverbrauch (LoT und DoT) in der mPCR Gruppe im Vergleich zu der historischen Kontrollgruppe signifikant niedriger. Eine Aufteilung nach Alter (Säuglinge vs. ältere Kinder) zeigte, dass bei Säuglingen sowohl der Antibiotika- als auch der Aciclovirverbrauch signifikant reduziert war, während dies bei älteren Kindern nur für den Aciclovirverbrauch zutraf. Ein signifikanter Unterschied in der Krankenhausaufenthaltsdauer wurde weder in der Subgruppenanalyse der ersten Studie noch beim Vergleich mit der historischen Kontrollgruppe der zweiten Studie festgestellt.

Zusammenfassend lässt sich festhalten, dass die Implementation des FA ME Panels als POCT einen schnelleren Erregernachweis oder -ausschluss sowie ein regelmäßiges, simultanes Testen auf ein breiteres Erregerspektrum ermöglichte. Nach dessen Einführung wurde eine signifikant höhere Anzahl viraler Erreger detektiert. Zudem führte der Nach-

weis von Viren, auf die früher nur selten getestet wurde, zu einem zunehmendem Bewusstsein, dass ein breiteres Spektrum viraler Erreger für eine Meningitis und Enzephalitis ursächlich sein kann. Unsere Ergebnisse suggerieren, dass die Einführung einer Liquor mPCR als POCT in einer Kinderklinik zu einer frühzeitigeren Optimierung der empirischen antiinfektiösen Therapie führen kann und somit zu einer Reduktion unnötiger Antibiotika- und Aciclovirtherapien bis hin zu einem kompletten Therapieverzicht. Eine signifikante Reduktion der Krankenaufenthaltsdauer wurde jedoch nicht beobachtet. Den Studien zufolge profitierte die Altersgruppe der jungen Säuglinge am meisten von der schnellen molekularen Diagnostik. Das FA ME Panel ist eine relativ teure diagnostische Methode [132]. Andere Strategien unter Verwendung von zielgerichteten Singleplex-PCRs als POCT (z.B. für HSV, Enterovirus und HHV-6) könnten kosteneffizienter und nach unseren Ergebnissen möglicherweise ausreichend sein, jedoch auch schwieriger umzusetzen. Zudem könnten weitere infektiöse Erreger unentdeckt bleiben.

Um das Panel effizienter und kosteneffektiver zu nutzen, bedarf es einer zielgerichteteren sowie strukturierteren Herangehensweise als bisher erfolgt. Hierfür könnte der großzügigere Einsatz des FA ME Panels (i.e. ohne Rücksprache mit dem pädiatrischen infektiologischen Team vor Einsatz der mPCR) auf Säuglinge im Alter von 1–12 Wochen beschränkt werden. Zudem kann eine kritische Patientenevaluation, welche auch den spezifischen Krankheitsverlauf sowie alle anderen vorhandenen Informationen (z.B. Begleitsymptomatik, Exposition, etc.) berücksichtigt, den Einsatz des Panels optimieren. Das FA ME Panel könnte ein nützlicher Test für junge Säuglinge mit Verdacht auf Sepsis oder Meningitis und/oder Enzephalitis sowie für ältere Kinder mit Verdacht auf Meningitis und/oder Enzephalitis sein. Allerdings, wie durch Ergebnisse der Studie unterstützt (z.B. falsch negative mPCR Ergebnisse, klinisch irrelevante HHV-6 Nachweise mit oder ohne bakterieller Koinfektionen außerhalb des ZNS), sollte die Interpretation der FA ME Panel Ergebnisse in Zusammenschau weiterer Parameter, wie z.B. Symptomatik und Ergebnisse anderer diagnostischer Tests erfolgen, wie auch vom Hersteller empfohlen wird [131]. Bei weiterhin hohem Verdacht auf eine bakterielle oder HSV-Infektion trotz eines negativen mPCR Ergebnisses oder Nachweises eines anderen Erregers sollten die empirische antiinfektiöse Therapie fortgeführt und weitere diagnostische Tests durchgeführt werden.

In Anbetracht dessen, dass beide Studien retrospektiv durchgeführt sowie nur ein einzelnes Krankenhaus eingeschlossen wurde, das Studienkollektiv relativ klein war und es nur

eine geringe Prozentzahl an positiven Testergebnissen gab, bedarf es weiterer prospektiver, multizentrischer Studien mit größerer Fallzahl, um die Auswirkungen der Einführung eines FA ME Panels als POCT in einer pädiatrischen Einrichtung weiterführend zu untersuchen.

Zusammengefasst ist die weitere prospektive Evaluation des FA ME Panels und die Optimierung seines Einsatzes erforderlich. Die Ergebnisse der Studien weisen jedoch bereits darauf hin, dass das FA ME Panel als POCT das Potenzial hat, ein nützlicher Test zu sein, um klinische Entscheidungen frühzeitig treffen zu können und das Patientenmanagement zu verbessern, insbesondere bei jungen Säuglingen.

4 Publication I

Clinical benefits of introducing real-time multiplex PCR for cerebrospinal fluid as routine diagnostic at a tertiary care pediatric center.

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Infection. 2019 Feb;47(1):51-58. doi: 10.1007/s15010-018-1212-7. Epub 2018 Sep 5.

5 Publication II

Comparison of antibiotic and acyclovir usage before and after the implementation of an on-site FilmArray meningitis/encephalitis panel in an academic tertiary pediatric hospital: a retrospective observational study.

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BMC Pediatr. 2020 Feb 5;20(1):56. doi: 10.1186/s12887-020-1944-2.

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Acknowledgement

I would like to formally express my deepest gratitude to all the people that have accompanied and supported me all along the way.

A special thanks goes to my supervisor Professor Dr. med. Johannes Hübner. Thank you for providing this interesting topic and supporting me throughout this process. You have always found time to offer guidance and inspire me to do my best work. I will hopefully be able to mentor others in the same capacity as you have for me, so thank you.

My sincere gratitude to Dr. med. Anna Eichinger. Your immense patience with me is deeply appreciated. The time you put into supporting me and giving me valuable advice was a sacrifice you did not need to make, however, you did find the time and that is something I will cherish forever. I now see you as my friend and someone I owe so much too.

Further, I would like to thank Dr. rer. nat. Melanie Meyer-Bühn and Dr. med. Tilmann Schober for your assistance and fruitful suggestions.

To my family and friends, thank you for being my constant source of encouragement. Thank you for your love and support all along the journey.