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VALUE OF MULTIPLEX PCR USING CEREBROSPINAL FLUID FOR THE DIAGNOSIS OF VENTRICULOSTOMY-RELATED MENINGITIS IN NEUROSURGERY PATIENTS

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Introduction

28 Nosocomial infections of the central nervous system are a serious
29complication of neurosurgical procedures and are associated with both severity of
30illness and exposure to invasive devices [1]. External ventricular drainage (EVD)
31catheters are frequently used in neurosurgical departments to monitor and treat
32intracranial pressure, to control transient hydrocephalus, and to prevent
33cerebrospinal fluid (CSF) fistulas after neurosurgical procedures.

34 The primary complication associated with EVD catheters is EVD-related
35ventriculo-meningitis [2]. It occurs in 2% to 27% of patients and exerts a relevant
36impact on morbidity and mortality rates among critically ill patients [2, 3]. The
37accurate and timely diagnosis of EVD-related infections in neurosurgical patients is
38highly important because the early availability of information about the causative
39pathogen is crucial for targeted antibiotic therapy and patient management (e.g.,
40removal of EVD) [4].

41 The diagnosis of EVD-related ventriculo-meningitis is based on a combination
42of clinical signs, laboratory values, and microbiological examination [2]. Cultural
43growth in CSF is obligatory for the detection of pathogens and for drug susceptibility
44testing; therefore, CSF culture is still the gold standard for the diagnosis of EVD-
45related ventriculo-meningitis [4]. However, obtaining results by CSF culture with the
46use of conventional microbiological methods requires one to two days. Therefore,
47nucleic acid amplification assays have been developed to overcome the long
48incubation periods of culture-based methods and to achieve increased sensitivity. A
49commercially available real-time polymerase chain reaction (PCR) assay, SeptiFast
50(SF, Roche Molecular Diagnostics, Mannheim, Germany), can detect 25 bacterial
51and fungal pathogens [5].

52 In this study, the clinical utility of SF in combination with the analysis of
53intrathecal interleukin 6 (IL-6) and lactate concentrations was compared to that of

54CSF culture for the diagnosis of EVD-related ventriculo-meningitis in patients in a
55neurosurgical intensive care unit (ICU).

56

57**Patients and Methods**

58*Setting*

59 The University Hospital Essen, Germany, is a 1300-bed tertiary care teaching
60hospital treating approximately 50,000 inpatients per year. The Department of
61Neurosurgery performs approximately 2,200 operative procedures per year and has
62a 10-bed ICU.

63*Study design*

64 We conducted a prospective observational study over one year from the
65beginning of October 2008 until the end of September 2009. We obtained 62 CSF
66samples from 42 consecutive neurosurgical ICU patients with possible ventriculitis
67related to EVD. CSF analysis including leucocyte cell count, concentrations of
68glucose, protein, IL-6, and lactate were performed in parallel to pathogen detection
69by SF and by routine microbiological examination of culture.

70*Inclusion and exclusion criteria*

71 All patients aged 18 or older admitted to the neurosurgical ICU with possible
72EVD-related ventriculo-meningitis and an indication for CSF collection were eligible
73for inclusion in the study.

74*Diagnosis of EVD-related ventriculo-meningitis*

75 A diagnosis of EVD-related ventriculo-meningitis was made if at least two of
76the following three criteria were met: (1) one or more new clinical signs of the
77disease, such as nuchal rigidity, headache, fever, or neurological deterioration
78(difference of ≥ 1 point in short form of the Scandinavian Stroke Scale with exclusion

79of deterioration due to primary disease) [6]; (2) increase of 100% or more in CSF cell
80count; or (3) detection of bacteria or fungi by CSF culture or PCR.

81*Analysis of CSF*

82 CSF was collected under sterile conditions by needle aspiration through a
83rubber port that is part of the closed drainage and monitoring system. Three times
84each week, CSF was collected routinely for cell count, culture, and biochemical tests
85for glucose, protein, and lactate concentrations. A portion (2 mL) of each sample was
86inoculated into a blood culture bottle (PEDS Plus/F, BACTEC 9240, Becton
87Dickinson, Heidelberg, Germany) supplemented with Fastidious Organism
88Supplement (FOS, Becton Dickinson). The bottle was incubated for as long as 7
89days at 36±1ºC under aerobic conditions. When a bottle was flagged as "positive" by
90the semi-automated BACTEC system, the bottle was removed from the instrument.
91An aliquot was collected for Gram staining and inoculated onto Columbia blood agar,
92chocolate blood agar, MacConkey agar and Brilliance Candida agar (all from Oxoid,
93Wesel, Germany). The solid media were incubated at 36±1ºC two days under aerobic
94conditions. Cultured organisms were identified by the semiautomated MicroScan
95WalkAway system (Siemens, Hilden, Germany).

96*Measurement of IL-6 concentrations*

97 IL-6 concentrations in CSF were determined using a solid-phase, two-site
98chemiluminescent enzyme immunometric assay (Immulit®, Siemens, Hilden,
99Germany), as previously described [7]. IL-6 results were available within 4 hours.

100*LightCycler Septifast Workflow*

101 Aliquots (1.5 mL) from the same CSF samples that were used for biomarker
102analysis and culture were subjected to mechanical lysis with ceramic beads in a
103MagNA Lyzer® instrument (Roche Molecular Diagnostics), as previously reported [5].
104The spectrum of species that can be detected by SF is stated in the following:

105*Escherichia coli, Klebsiella pneumonia*/*oxytoca*, *Serratia marcescens*, *Enterobacter*
106*cloacae*/*aerogenes*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter*
107*baumannii*, coagulase-negative *Staphylococcus* species, *Staphylococcus aureus*,
108*Streptococcus pneumoniae*, *Streptococcus* spp., *Enterococcus faecium* and *E.*
109*faecalis, Candida albicans, C. tropicalis*, *C. parapsilosis*, *C. krusei, C. glabrata* and
110*Aspergillus fumigatus*. Typical pathogens which can cause community- or hospital-
111acquired meningitis such as *Listeria* spp., *Neisseria meningitis*, *Propionibacterium*
112spp. and anaerobes are not covered by the SF master list. The manufacturer has
113designed the DNA assay software to be less sensitive to coagulase-negative
114staphylococci (CoNS) and *Streptococcus* spp. and not to interpret the results as
115positive if the cut-off values for these pathogens are exceeded in the twentieth PCR
116cycle or later [5].

117 The working hours of the technical laboratory personnel are from 7.30 am to
1184.00 pm. The SF assay is performed in the laboratory from Monday to Friday and not
119at weekends. Receiving same-day-results (from sample collection on ward to
120obtaining SF results) is only possible if the sample arrives in the laboratory before
12111.00 am. Samples that arrive later can be processed and the PCR can be run over
122night, so that the results are available the next morning.

123*Study definitions*

124 A CSF sample was considered positive when a bottle was flagged as positive
125and we could identify a pathogen with conventional microbiological methods. If a
126culture tested positive for common skin pathogens, especially for CoNS, repeated
127evidence from two separate samples was required to establish the diagnosis of CSF
128infection. If only one CSF culture tested positive, the result was deemed to be due to
129contamination. SF results were considered positive and concordant when this test
130identified the same microorganism as the CSF culture. It was considered positive and

131disconcordant when it identified a pathogen different from the one in CSF culture and
132negative when it did not identify any microorganism.

133*Statistics*

134 The diagnostic accuracy of IL-6 and lactate for SF and CSF culture was
135compared by using receiver operating characteristic curve (ROC) analysis. The
136results were given as area under the curve (AUC) with a 95% confidence interval
137(CI). Statistical analysis was performed with GraphPad Prism, version 5.02
138(GraphPad Software, Inc, San Diego, CA, USA).

139

140**Results**

141 The mean age (± SD) of the 42 included patients (24 men, 18 women) was
14256.0±15.0 years. All patient characteristics are shown in Table 1.

143 In 17 (27%) CSF samples a pathogen was detected by both, SF or CSF
144culture. The results of CSF culture and SF were concordant for 57 (92%) samples:
14540 (65%) negative; 17 (27%) positive (Table 2). The remaining 5 (8%) samples gave
146discordant results: 4 (6%) samples tested positive by CSF culture and negative by
147SF, whereas 1 (2%) tested negative by CSF culture and positive by SF. CSF culture
148yielded a higher positive rate than SF (CSF culture, 21; SF, 18). SF exhibited a
149sensitivity of 80.1%, a specificity of 97.6%, a positive predictive value (PPV) of
15094.4%, and a negative predictive value (NPV) of 90.9% compared to traditional
151culture. The diagnostic accuracy of IL-6 and lactate for SF and CSF culture was
152compared by ROC analysis (Table 2). The largest area under the curve (AUC) in the
153detection of pathogens with SF was found for IL-6 (0.90; 95% CI, 0.83–0.98)
154compared to culture (0.70; 95% CI, 0.46–0.80). The AUC for lactate was 0.77 (95%
155CI, 0.63–0.93) with SF and 0.65 (95% CI, 0.50–0.80) with culture. When the cut-off
156levels were set at the closest point to 100% sensitivity and 100% specificity, the

157concentration level for pathogen detection by SF was 6559 pg/mL for IL-6, with a
158sensitivity of 93.8%, a specificity of 76.6%, a PPV of 59%, and a NPV of 97%. In
159contrast, the optimal cut-off for lactate was found to be 4.2 mmol/L, with a calculated
160sensitivity of 86.7%, a specificity of 68.9%, a PPV of 30.2%, and a NPV of 88.2%.

161 The distribution of identified microorganisms obtained by both methods is
162presented in Table 3. No polymicrobial infections were detected. Three samples
163tested positive for CoNS by culture but negative by SF, whereas only one sample
164tested positive for CoNS by SF but negative by CSF culture. According to the study
165definitions, three of the four CoNS spp. detected by CSF culture were interpreted as
166contaminations. A *Corynebacterium* spp., which is not covered by the SF panel, was
167detected by culture in one CSF sample. The therapeutic consequences of positive
168SF results were as follows: removal of EVD catheter in 6 cases, initiation of antibiotic
169therapy in 6 cases, continuation of antibiotic therapy in 7 cases, changes in treatment
170regimens in 4 cases, and additional intrathecal application of antibiotics in 3 cases.
171One patient died before the SF result was available. The time-to-result of the
172diagnostic procedures (SF, time-to-positivity of BACTEC bottles, Gram stain and
173species identification) is shown in Table 3. In 94% of positive SF samples the results
174were obtained on the same day of sample collection whereas the overall mean of the
175time-to-positivity of BACTEC bottles was 21.6 hours. The mean time for species
176identification by conventional methods was 54.8 hours.

177

178**Discussion**

179 Clinical signs in ICU patients with EVD-related ventriculo-meningitis are often
180unspecific because such signs may be a manifestation of the underlying disease or a
181complication of ICU treatment [8]. In addition, the interpretation of pathologic CSF
182laboratory values cannot be used as a stand-alone marker (e.g., cell count, protein,

183glucose) for the diagnosis of an EVD-related infection [9, 10]. Such laboratory values
184can be the result of postoperative local inflammation caused by blood or tissue
185breakdown products, sutures, or chemicals, or perhaps by small bacterial inocula [11,
18612]. Even the results of Gram staining of the CSF can exhibit high specificity (99%)
187but low sensitivity (18%) in this specific type of patients [13]. The use of
188microbiological culture methods is well established in the diagnosis of infections of
189the central venous system. However, drawbacks are the necessary incubation time
190and the lack of sensitivity. In addition, even the detection by culture of pathogens in
191the CSF is not definitive because of potential contamination or colonization of the
192catheter by bacteria [14].

193 This study demonstrated that the SF assay can be used to identify pathogens
194in CSF within one day. In 91% of the samples tested, the results of SF were
195concordant with those achieved by standard culture method. The two methods
196differed in the detection of CoNS, and in one case a *Corynebacterium* spp. was
197cultured. These differences can be explained by the fact that the sensitivity of the SF
198assay in detecting CoNS and streptococci was set by the manufacturer to be
199relatively low [5]. As has been demonstrated by extensive reviews from various
200authors, gram-positive cocci (mainly staphylococci) consistent with skin flora are the
201most common pathogens involved in EVD-associated infections [2, 4, 15].

202 The mean time-to-positivity (TTP) of positive BACTEC CSF cultures was 21.6
203h. This TTP is higher than the TTP of blood cultures (BC) described in the literature
204[16]. One reason for this might be that in more than half of the cases commensal
205bacteria, e.g. CoNS, were detected. In those cases, in which CoNS were considered
206contaminants, the TTP of the BACTEC bottles was prolonged. In these cases SF
207was negative. To the best of our knowledge no study to date has systematically
208analysed the TTP of CSF cultures.

209 The mean time for species identification was 54.8 h by BACTEC CSF cultures
210whereas SF results were, in 94.8% of cases, obtained on the same day. These
211detection times are in line with a study from Bloos et al. who compared SF with BC
212results from 245 patients with suspected sepsis and found that the median time for
213positive BC results was 68.8 h and, under optimal logistical conditions, 7.2 h for SF
214[17]. With the use of MALDI-TOF MS the time to identification could be reduced and
215in cases with clinically relevant pathogens combined with low TTP, the species
216identification might be possible in less than 24 h [18].

217 Two studies evaluated the use of in-house PCR assays of CSF to diagnose
218ventricular drainage-related meningitis, and both found that the PCR assays used
219were sensitive and rapid [19, 20]. Studies using 16S rDNA sequencing for direct
220universal pathogen detection in CSF have also been performed. However, neither
221study evaluated the performance of a commercially available PCR assay in CSF or
222tested the efficiency of such a system in combination with measurements of
223intrathecal biomarkers.

224 Another important finding of our study was its greater diagnostic accuracy in
225measuring intrathecal IL-6 than lactate concentrations. Only a few studies have
226analysed the concentrations of these two biomarkers in the CSF of neurosurgery
227patients with EVD. Schade and co-workers found that IL-6 concentrations on days 2
228and 3 were higher in patients with EVD-related infection than in those without such
229infection; however, conditional logistic regression analyses of 10 case-control pairs
230detected no significant differences in IL-6 concentrations between the groups [13].
231Two other studies reported that the IL-6 concentration in CSF is an early and useful
232marker for the diagnosis of EVD-related infection [7, 21].

233 Mostly in line with our results, the findings of a small study involving 16
234patients with intraventricular hemorrhage and EVD demonstrated that CSF infection

235could be detected in 3 patients with a lactate cut-off level of 4 mmol/L [22]. In
236contrast, a very recent retrospective study by Walti and colleagues examined the
237characteristics of 48 patients with EVD-associated infection over a period of 12 years
238[23]. None of the CSF values, including lactate concentrations, reached an accuracy
239of AUC ≥ 0.70. That study did not evaluate IL-6 concentrations.

240 We propose a diagnostic algorithm for neurosurgery ICU patients with possible
241EVD-related ventriculo-meningitis, such as the following: sample CSF, perform CSF
242profile of all variables, and send at least 2 mL CSF to the microbiological department
243for CSF culture. If pathologic levels of CSF parameters are present, especially IL-6
244(>6559 pg/mL) or lactate (>4.2 mmol/L), the microbiology department should also be
245requested to perform a SF assay by using 1.5 mL of the CSF sample that has
246already been collected. Our findings suggest that the possibility of identifying the
247causative pathogen(s) in this sample is very high. This approach may allow a more
248rapid means of confirming the clinical suspicion of EVD-related meningitis by
249laboratory values and of identifying the probable causative agent at an early stage.
250Early identification might be important for the administration of appropriate or
251perhaps better species-adapted antimicrobial therapy.

252 Several factors may limit the interpretation of the results of this study. First, the
253study was performed at a single center, and the number of enrolled patients is
254relatively small. Thus, the results should be interpreted with caution and cannot be
255generalized. Second, we did not compare direct plating of CSF on solid media with
256SF and BACTEC CSF culture. With this approach a differentiation between
257contamination and infection and the comparison of the time-to-result of the different
258detection methods may also have also been possible. Third, SF is incapable in
259detecting detect anaerobes such as propionibacteria. Also the culture conditions did
260not allow the growth of anaerobic bacteria. In addition, in this observational study we

261did not assess the overall benefits of SF for the patient's outcome. Thus, future 262studies should focus on the relevance of these fast and accurate microbiological 263findings for the patients and should determine whether the PCR approach has 264implications for antibiotic usage orlength of stay in the ICU. Furthermore, a cost-265benefit analysis of the use of SF assays should be performed.

266

267**Conclusions**

268 SF is suitable for the identification of microorganisms in CSF samples from 269neurosurgery ICU patients with EVD. This rapid assay may assist physicians by 270providing information that can allow the timely initiation or adjustment of appropriate 271antibiotic therapy. To the best of our knowledge, this is the first study to test the 272clinical utility of a multiplex PCR assay for detecting pathogens in CSF in 273combination with the determination of concentrations of intrathecal biomarkers such 274as IL-6 and lactate.

275

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277None.

278

279**Conflict declaration**

280P.-M. Rath and J. Steinmann have been on the speaker's bureau for Roche 281Diagnostics Germany.

282

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