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BRIEF REPORT

Microbiological Outcomes Associated With Low Leukocyte Counts in Cerebrospinal Fluid

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The significance of low leukocyte counts in cerebrospinal fluid (CSF) remains unclear. We performed a 2-year retrospective study to examine microbiological outcomes associated with CSF leukocytes at 6–10/mm^3. Of the 178 samples examined, we detected positive results for 11 samples, including 5 cases of tick-borne encephalitis virus infection.

Keywords. cerebrospinal fluid; encephalitis; leukocytes; meningitis; tick-borne encephalitis.

Neuromeningeal infections are diagnosed frequently and are associated with significant morbidity and mortality [1]. Outcomes associated with these infections depend on the rapid diagnosis and initiation of treatments [2, 3]. Analysis of cerebrospinal fluid (CSF) remains the main method to assess the etiology of central nervous system (CNS) disease. A full examination of CSF typically includes total protein, glucose, gram stain, and white blood cell (WBC) count. Normal CSF WBC counts are ≤5/mm^3, and counts >5/mm^3 should be investigated as indicated by guidelines [4, 5].

Leukocytosis (>10/mm^3) suggests meningitis or meningoencephalitis, and it is typically followed by the rapid initiation of appropriate therapy. In contrast, normal WBC counts are rarely detected in patients with CNS infection. Several studies have particularly focused on meningitis without the elevation of the CSF WBC count or compared patients with normal and elevated (>5/mm^3) CSF WBC counts [6–8]. Immunosuppression can decrease the meningeal inflammatory response or delay the elevation of CSF WBC counts. Other etiologies can induce a moderate meningeal inflammatory response or similar postseizure condition [9, 10]. However, in our experience, the diagnostic significance of moderately elevated CSF WBC counts (ie, 6–10 leukocytes/mm^3) in adults is unclear for most physicians in daily practice.

To address this issue, we performed a 2-year retrospective study of suspected meningoencephalitis or meningitis; specifically, we identified cases of documented neuromeningeal infection associated with CSF WBC counts at 6–10/mm^3.

METHODS

We carried out a retrospective, descriptive multicenter study at Hôpitaux Civils de Colmar (HCC) and University Hospitals of Strasbourg (UHS) that included patients >15 years of age who were hospitalized from 01/01/2017 to 12/31/2018 at UHS or from 05/31/2016 to 06/01/2017 at HCC and who underwent lumbar puncture to diagnose suspected neuromeningeal infection with resulting CSF WBC counts of ≥6/mm^3 and ≤10/mm^3. We excluded cases in which the CSF analysis did not include a full microbiological assessment; the latter includes bacterial cultures and polymerase chain reaction (PCR)–based evaluations to detect herpes simplex virus (HSV) 1/2 and varicella zoster virus (VZV).

Cases with hemorrhagic CSF were excluded if they revealed a WBC count of ≤5/mm^3 after subtraction of 1 leukocyte for every 1000 red blood cells. Blood cultures and bacterial or viral serologies were evaluated if available.

CSF leukocytes (total and cell differentials) were subjected to May-Grunwald-Giemsa staining and enumerated in a Kova slide counting chamber. PCR was performed using Argene/Biomerieux R-gene kits. CSF protein glucose and bacterial cultures were performed using standard procedures. In addition, we collected retrospective clinical data from patients' medical records, including histories of primary or acquired immunosuppression (chemotherapy during the last 5 years, immunosuppressive treatment, and corticosteroid therapy at doses >20 mg/d).

Patient Consent Statement

The study was approved by the Ethical Committee of Medicine Odontology and Pharmacy Faculties and Hospitals (UHS; No. FC-2020-10) with a waiver of consent.

RESULTS

A total of 631 patients met the study criteria; 453 were excluded due to hemorrhagic CSF samples (n = 19) or incomplete

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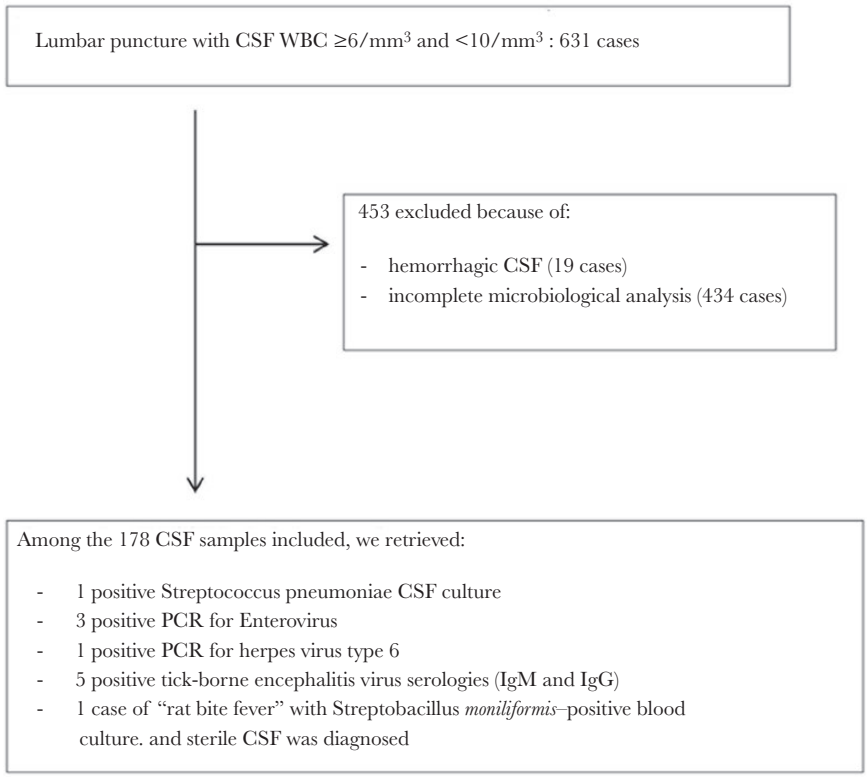

Figure 1. Study flowchart. Abbreviations: CSF, cerebrospinal fluid; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction; WBC, white blood cell count.

microbiological analysis (n = 434) (Figure 1). All CSF samples were subjected to direct gram staining and culture. Patients were most commonly excluded because of a lack of PCR data for HSV1/2 and VZV (434 of 453 cases).

Among the 178 cases enrolled in the study, the average age (range) was 50.6 (15–97) years, and the study included 95 women and 83 men (sex ratio M/F = 0.87). The median CSF total protein (range) was 0.43 (0.11–14.34) g/L, and the median glucose level (range) was 0.64 (0.06–6.61) g/L. Among the nonstandard microbiological investigations, PCR targeting enteroviruses was performed in 105 cases (59%). Serology for tick-borne encephalitis virus (TBEV) was assessed in 9 patients (from 2016 to February 2018, Serion classic TBE virus kit immunoglobulin M [IgM] and immunoglobulin G [IgG] Virion/Serion; and since February 2018, TBE Enzygnost IgM and IgG Siemens) according to the seasonal distribution of the infection in our country from spring to the beginning of autumn (5%) (Table 1).

Of the 11 cases (5.6%) associated with microbiological diagnoses, we identified 1 CSF culture-positive case of *Streptococcus pneumoniae*, 4 PCR-positive tests for viruses, including *Enterovirus* (n = 3) and herpesvirus type 6 (n = 1), and 5 positive TBE blood tests (IgM and IgG). One case of "rat bite fever" was diagnosed based on *Streptobacillus moniliformis*–positive blood cultures with a negative LightCycler SeptiFast (LCS) test. Among these 11 patients, the main clinical symptoms at the time of admission were headache (n = 6, 55%) and fever (n = 8, 73%). Other symptoms included neck stiffness (n = 3, 27%) and disturbances of consciousness and confusion (n = 8, 73%). Less commonly reported systems included focal deficits (n = 1, 9%) and seizures (n = 1, 9%). Two patients underwent brain magnetic resonance imaging (MRI); an abnormal result was reported in the case of pneumococcal meningoencephalitis. Demographic characteristics, clinical symptoms, and CSF analyses are summarized in Table 1. In total, 24 of 178 patients (13.5%) were immunocompromised. None of these 24 patients had a positive microbiological result.

DISCUSSION

Our study focused on the analyses of CSF specimens with slightly high leukocyte counts (≥6 and ≤10 cells/mm^3); moderate leukocytosis is not rare, and it can cause confusion in clinical practice, though counts >5/mm^3 should be investigated as indicated by guidelines [4, 5]. The results of this study revealed that CSF leukocyte counts between 6/mm^3 and 10/mm^3 are only rarely associated with a documented infection; among 178 cases with suspected meningitis or encephalitis (as underscored by the complete microbiological analysis performed in all cases), we identified only 1 positive CSF bacterial culture. Classically,

Table 1. Demographic Characteristics, Clinical Symptoms, and Microbiological Diagnoses

Demographic Characteristics		
Female sex, No. (%)		95 (53.3)
Age, mean (range), y		50.6 (15–91)
CSF total protein, median (range), g/L		0.58 (0.11–14.34)
CSF glucose, median (range), g/L		0.73 (0.06–6.61)
Microbiological diagnoses		
1. TBEV	5 cases	
	Clinical symptoms	
	Fever	5
	Headache	3
	Psychomotor slowing	4
	Neck stiffness	1
	Confusion	1
	Radiculitis	1
	CSF protein, median (range), g/L	0.64 (0.43–0.86)
2. *Enterovirus*	3 cases	
	Clinical symptoms	
	Fever	1
	Headache	2
	Neck stiffness	1
	CSF protein, median (range), g/L	0.39 (0.18–0.54)
3. *S. pneumoniae*	1 case	
	Clinical symptoms: seizure, coma, focal neurological impairment, fever	
	CSF total protein, g/L	2.71
4. Human herpesvirus-6	1 case	
	CSF total protein, g/L	0.36
	Clinical symptoms: psychomotor delay, seizure	
5. Rat bite fever	1 case	
	CSF total protein, g/L	0.18
	Clinical symptoms: fever, headache, rash, psychomotor delay	

Abbreviations: CSF, cerebrospinal fluid; TBEV, tick-borne encephalitis virus.

neuromeningeal infections associated with mild pleocytosis are associated with poor outcomes, especially in cases of invasive pneumococcal infection [11]. In our case of CSF culture-proven infection, the evolution was indeed rapidly fatal.

Immunosuppression, particularly primary or acquired cellular immunodeficiency, is usually associated with mild pleocytosis. Among these situations, HIV infection is readily accompanied by HSV encephalitis without pleocytosis [12, 13]. However, among the 24 immunocompromised patients, we did not report any documented infections.

Viral agents were detected frequently, and we found an unexpectedly high rate of positive TBE serology.

These results are consistent with findings from previous studies in which normal CSF leukocytes counts were reported for viral infections of the CNS. In a series of 379 patients with CSF who tested positive for HSV by PCR, Cag et al. described 14 cases in which the CSF WBC counts were within normal limits when lumbar puncture was performed 0–24 hours after admission [7]. In our study, none of the samples was PCR-positive for HSV or VZV, and 5 samples were positive for TBE. Günther et al. [14] compared 85 patients with TBE with 85 controls. The control group included 27 patients with enteroviral infection, 12 patients with HSV1/2 infection, 1 patient with cytomegalovirus infection, and 24 patients with unknown etiologies. Patients with TBE had a significantly lower CSF WBC count than those without TBE during the acute stage, which may explain the high proportion of TBE cases in our study. The mean age of the patients in our study was similar to that in other studies but lower than that in the primary HSV1/2 encephalitis cohorts.

Enteroviruses represented a substantial proportion of the pathogen-positive results (27%). The 3 CSF samples that tested positive for enteroviruses were from young adults (22, 24, and 33 years old) and were associated with increased levels of total protein (0.54, 0.39, and 0.18 g/L, respectively; mean, 0.37 g/L). Enteroviruses are the most common cause of meningitis, most notably among pediatric patients; the literature with respect to results is less certain. Troendle et al. [8] described 2 of 124 cases of *Enterovirus*-associated meningitis without elevated CSF leukocyte counts. In another series, Ahlbrecht et al. [15] reported similar findings in 15% of adults with CNS enteroviral infection.

We identified only 1 case of CNS bacterial infection associated with a low CSF WBC count. Interestingly, Hase et al. [16] presented a review of 26 cases of bacterial neuromeningeal infections without elevated CSF WBC counts. The main pathogens identified in these cases were *Streptococcus pneumoniae* (n = 10) and *Neisseria meningitidis* (n = 11). One of the explanations provided was that the CSF samples were obtained from lumbar puncture procedures that were carried out early during the course of the disease; leukocytosis (>10/mm^3) was observed in cases in which a second lumbar puncture was performed. This hypothesis was corroborated by the findings of Costerus et al. [17], in which the initial CSF cell count was normal in 30 of 1382 patients. Literature on bacterial meningitis without pleocytosis in adults is scarce [17, 18]. Polk and Steele [19] reported 7 cases of positive CSF culture among 261 cases of pediatric meningitis without pleocytosis, accounting for 0.5%–12% of all cases of bacterial meningitis. The series presented by Troendle et al. [8] included similar rates of positivity for *N. meningitidis* (n = 25), *S. pneumoniae* (n = 22), and *H. influenzae* (n = 17) associated with negative results from CSF; interesting, no leukocytosis was observed in 4 of 37 CSF samples from second lumbar punctures. Our study included only 1 case of pneumococcal meningoencephalitis; this case featured a comparatively high level of CSF total protein (2.71 g/L), which could be an important factor associated with this diagnosis, as described elsewhere [20].

Our case series included a relatively high proportion of CSF samples with positive serologies indicative of TBEV infection. France is a European country with a low incidence of TBE, and the Alsace region of Northeastern France represents the westernmost limit of viral spread from the high-endemic

regions of Baden-Württemberg and Bavaria [21]. There are typically only 10–20 cases of TBE in the Alsace region annually [22]. However, the epidemiology of TBE is evolving in Europe, with possible cases reported in the United Kingdom [23] and the Netherlands [24], in addition to greater numbers of cases reported in the Auvergne Rhone region of France [25], and the marked increase of TBE cases in France in 2016 highlights the possibility of epidemic flares in these regions [26]. Thus, reporting of all cases of TBE became mandatory in France in 2020 [22]. TBEV is an RNA virus of the genus *Flavivirus* transmitted by tick bites or, rarely, by the consumption of unpasteurized dairy products from infected cattle; humans are incidental hosts. Biphasic disease typically results after a 7–14-day incubation period. The viremic phase presents as a flu-like illness; after some symptomatic improvement, some patients develop a neurologic phase, which can include meningitis, meningoencephalitis, myelitis, and meningoradiculitis [22, 26, 27, 28]. Diagnosis of TBE relies on the results of CSF analyses that include leukocytosis and virus-specific serology or PCR, as per the European Centre for Disease Prevention and Control criteria [29]. The presence of specific TBEV serum IgM and IgG antibodies or seroconversion is the best method in association with compatible clinical symptoms to diagnose TBE. Viral RNA is rarely detected by TBEV-specific RT-PCR in blood samples during the first viremic phase. RT-PCR methods do not contribute to diagnosis during the second neurologic phase [29]. The clinical symptoms reported by our patients are in agreement with those reported in the literature [30]. Of interest in this study, CSF analysis in 669 patients comprising adults and children with TBEV infection revealed CSF leukocyte counts exceeding 10 leukocytes/mm^3 in all patients, including mean counts (range) of 129 (10–896) leukocytes/mm^3 in children and 80 (11–1268) leukocytes/mm^3 in adults [30]. As part of a large retrospective study, Zavadska et al. enrolled 1973 confirmed cases of TBE that were diagnosed between 1973 and 2016; high CSF WBC counts were reported in 97.5% of these cases, but slightly elevated CSF WBC was not distinguished [31]. In our study, we identified 5 cases of TBE associated with low CSF WBC counts. The mean CSF total protein levels among these cases was 0.67 g/L, with a range of 0.43–0.86 g/L.

There are several limitations to our study. First, the design of the retrospective study did not facilitate evaluation and correlations with relevant data, including lactate levels in the CSF and/or the ratios of serum to CSF glucose. Another limitation of the study was related to the high number of patients excluded because of incomplete microbiological assessments. These criteria were introduced to avoid the inclusion of data for CSF analyses related to the lumbar puncture control of noninfectious conditions, such as demyelinating diseases or dementia. In both hospitals, viral PCR is systematically performed for HSV and VZV in cases of suspicion for meningitis or meningoencephalitis, although *Enterovirus* PCR is systematically performed at HCC and less frequently performed at UHS, explaining why enterovirus PCR was performed for only 105 patients. However, we cannot exclude the possibility that some of the excluded cases were not related to an actual infection of the neurologic system. It is important to remember that some viral etiologies, such as enteroviruses and TBEV, classically exhibit a seasonal distribution. Some CSF samples analyzed in our study were sampled in winter when TBEV does not circulate. Furthermore, many physicians are not fully cognizant of the emergence of TBEV infection in our region; this may explain why so few tests for TBEV serology were performed compared with those focusing on other viral diagnoses.

Overall, our study focused on moderate elevations in CSF leukocyte count. Our results underscore the fact that one cannot use this finding to distinguish bacterial from viral infection, or to rule out infection overall. The degree of leukocytosis needs to be evaluated together with other relevant CSF and clinical data. Bacterial cultures and viral PCR and serologies should be carried out in cases in which clinical symptoms suggest CNS infection; TBEV serologies should always be considered in endemic regions as well as in those that may be experiencing an emergence of this disease, particularly during the epidemic season [22, 25, 31, 32].

CONCLUSIONS

Analysis of CSF remains a key element of the diagnostic approach to suspected neuromeningeal infections. The results of our study reveal that most patients with CSF WBC counts of 6–10/mm^3 were not diagnosed with an infection. Only 5.6% of the 178 examined patients were diagnosed in this manner. Nevertheless, slightly elevated CSF WBC counts may be indicative of viral infections, notably those associated with TBEV in TBEV-endemic regions.

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Potential conflicts of interest . The authors have no conflicts of interest to declare. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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