No Evidence of Viral Coinfection in Cerebrospinal Fluid From Patients With Community-Acquired Bacterial Meningitis

TO THE EDITOR—Bacterial meningitis is a severe disease with substantial mortality and morbidity despite the availability of effective antimicrobial agents and adjunctive therapies [1, 2]. Recently, a study involving adults with community-acquired bacterial meningitis in Malawi showed that coinfection with Epstein Barr Virus (EBV) occurred in 79 of 149 bacterial meningitis patients (53%) and that EBV load was associated with higher mortality [3]. Coinfection with cytomegalovirus (CMV) was detected in 7% of patients. A high proportion of patients (77%) in this study were human immunodeficiency virus (HIV) positive, which was found to have a strong influence on the rate of coinfection with EBV and CMV. The aim of the current study was to establish whether herpes viruses and other neurotropic viruses could be detected in the cerebrospinal fluid (CSF) of adult bacterial meningitis patients in a Dutch population with a low HIV infection rate.

We analyzed CSF samples from a random subset of bacterial meningitis patients included in a nationwide prospective cohort [4]. Patients were included if they had community-acquired bacterial meningitis confirmed by CSF culture or if CSF results showed at least 1 individual predictor of bacterial meningitis, defined as a glucose level of <34 mg/dL, a protein level of >220 mg/dL, or a leukocyte count of >2000 cells/μL [5]. Informed consent was obtained from all participating patients or their legally authorized representatives. CSF was stored locally at −80°C, after which it was brought to the Academic Medical Center (Amsterdam, the Netherlands), where samples were thawed once, spun down, aliquoted, and again stored at −80°C until analysis. Nucleic acid was extracted by automated extraction (MagnaPure, Roche Diagnostics), and reverse transcription using random hexamers was performed. Five microliters of reverse transcription reaction was subsequently used to detect EBV, CMV, herpes simplex virus 1 and 2, varicella-zoster virus, adenovirus, enterovirus, human parechovirus, and human herpesvirus types 6 and 7 simultaneously in an internally controlled 4-tube real-time multiplex assay.

CSF was available for 204 of 642 bacterial meningitis patients (27%) included in the cohort [4]. Polymerase chain reaction (PCR) was performed on 56 random CSF samples, derived from the diagnostic lumbar puncture. Most of these 56 patients had classical symptoms and signs of bacterial meningitis, and CSF examination showed at least 1 individual predictor of bacterial meningitis in 49 patients (88%; Table 1) [5]. Causative bacteria were identified by CSF culture in 51 patients, which yielded *Streptococcus pneumonia* in CSF from 42 patients (72%), *Neisseria meningitidis* in CSF from 3 (5%), *Haemophilus influenza* in CSF from 2 (4%), and *Staphylococcus aureus*, *Streptococcus oralis*, and *Streptococcus agalactiae* each in CSF from 1 (2%). In 5 patients with negative results of CSF cultures, blood culture showed *Streptococcus pneumoniae* in 2 and *N. meningitidis* and *S. agalactiae* each in 1. All patients with negative results of CSF cultures had at least 1 individual CSF predictor of bacterial meningitis [5]. During the course of illness, 5 patients (9%) developed herpes simplex stomatitis, and 1 (4%) developed herpes zoster. Real-time PCR was
performed in all 56 samples, and results were negative for all tested viruses.

We did not identify viral coinfections in the CSF of patients with community-acquired bacterial meningitis in the Netherlands on admission. This finding is in contrast to the Malawian study among bacterial meningitis patients, which found frequent coinfection with EBV and CMV [3]. It was suggested that viral infections stress the host immune response, leaving the patient more susceptible to secondary viral and bacterial infections [6, 7]. This would increase the risk of bacterial meningitis and of having an unfavorable outcome. Alternatively, it was suggested that EBV detected in the study originated from latently infected lymphocytes entering the CSF from the bloodstream because of the breakdown of the blood-brain barrier during bacterial meningitis [3]. This mechanism may explain the differences between our study and the Malawi study, as all our samples were spun down and the supernatant was used for PCR. Therefore, latently infected cells did not influence the PCR results. The Malawi study did not describe whether CSF was spun down, and therefore it is unclear whether this explanation is valid. Differences in causative microorganisms between studies may also influence the risk of viral coinfection. The majority of patients in both cohorts were infected with S. pneumoniae and N. meningitidis. However, although Escherichia coli and Salmonella species were relatively frequent in the Malawi study [8], these pathogens were not identified in our population. In conclusion, we show that viral coinfection does not play a substantial role in HIV-negative Dutch adults with bacterial meningitis.

### Notes

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