Vascular endothelial growth factor (VEGF) is a potent vascular permeability factor and a mediator of brain edema. To assess the role of VEGF during bacterial meningitis, VEGF was measured in cerebrospinal fluid (CSF) and blood of 37 patients with bacterial meningitis and 51 control patients, including 16 patients with viral meningitis. Circulating VEGF levels were similar in bacterial meningitis patients and control patients. VEGF\textsubscript{CSF} was detected in 11 (30\%) of 37 of bacterial meningitis patients (range, 25-633 pg/mL) but in none of the control patients. The median VEGF index was 6.2 (range, 0.6-42), indicating intrathecal production. Median CSF cell counts, protein levels, and CSF:serum albumin ratios were higher for patients with detectable VEGF\textsubscript{CSF}, although the difference was not statistically significant. VEGF immunoreactivity in autopsy brain specimens was found in the inflammatory infiltrate of patients with bacterial meningitis. These results indicate that inflammatory cells secrete VEGF during bacterial meningitis and that VEGF may contribute to blood-brain barrier disruption.

Bacterial meningitis is associated with serious morbidity and mortality in both children and adults. Infection of the cerebrospinal fluid (CSF) causes a severe inflammatory reaction, mediated by bacterial products and host cytokines. This inflammatory reaction compromises the function of the blood-brain barrier (BBB), resulting in the exudation of plasma proteins and development of vasogenic brain edema, which contributes to cerebral dysfunction and brain damage. Vascular endothelial growth factor (VEGF), a 46-kDa glycosylated homodimeric protein, is a regulator of angiogenesis and a potent inducer of vascular permeability [1]. VEGF is implicated in the pathogenesis of brain edema related to ischemia, trauma, and tumors [2]. To assess the role of VEGF in the pathophysiology of bacterial meningitis, VEGF levels were measured in CSF of patients with bacterial meningitis and were compared with those of patients with viral meningitis and nonmeningitis control patients. In addition, immunohistochemical analysis of brain samples obtained by autopsy from patients who died of bacterial meningitis was used to identify the cellular source of VEGF\textsubscript{CSF} and to examine the expression of the VEGF receptors’ fms-like tyrosine kinase (Flt)-1 and fetal liver kinase-1/kinase insert domain-containing receptor (Flk-1/KDR).

**Patients and Methods**

Patients >1 month of age with bacterial meningitis diagnosed between January 1998 and January 2000 were eligible for the study. Thirty-seven patients were included, at the following centers: University Medical Center (UMC), Diakonessen Hospital, and Mesos Medical Center, Utrecht, and St. Antonius Hospital, Nieuwegein, The Netherlands; Departments of Neurology and Hematology and Oncology, University Hospital, Innsbruck, Austria.
[WBC] count >2000 cells/μL, CSF glucose level <1.9 mmol/L, and CSF/serum glucose <0.23 or CSF protein >2.2 g/L) [3]. Paired control samples of CSF and plasma or serum were obtained from 16 patients with viral meningitis, 17 patients who underwent spinal anesthesia for urologic or orthopedic procedures, and 18 patients presenting with fever in the emergency room, for whom a diagnosis of meningitis was excluded by lumbar puncture.

Autopsy cases and controls. The autopsy registration of the UMC (1990–1999) was searched for diagnoses of bacterial meningitis. Autopsy specimens obtained from 10 male and 8 female patients, with a median age of 31 years (range, 2 months–90 years), were recovered. All patients had a positive CSF culture or purulent CSF and additional pathologic signs of bacterial meningitis. Pathologic abnormalities ranged from mild brain edema and presence of inflammatory infiltrate to severe brain edema, lysis of neurons, ventriculitis, choroiditis, and abscess formation. The control group (11 male and 5 female patients) had a similar age distribution, with a median of 38 years (range, 1 month–83 years). No patients with brain tumors, trauma, or infection or other cerebral diseases were included in the control group, because increased VEGF expression is known to be associated with these disorders [2]. However, cerebral hypoxia preceding death cannot be excluded. Causes of death in the control group were as follows: cardiac failure (n = 4), respiratory failure (n = 2), hemorrhagic shock (n = 4), multiple organ failure (n = 1), hemolytic uremic syndrome (n = 1), neoplastic disease (n = 1), no definite cause (n = 1), and sudden infant death syndrome (n = 2).

Laboratory studies. Blood and CSF samples were collected at the time of admission and were centrifuged immediately (1700 g at 4°C), aliquoted, and stored at −70°C. Samples were examined for WBC count and levels of glucose, total protein, and albumin. Levels of VEGF in plasma and CSF were measured by ELISA (Quantikine; R&D Systems). For values below detection, the lower limit of detection in our hands was used for statistical analysis (25 pg/mL). To estimate intrathecal production of VEGF, the VEGF index was calculated analogous to the IgG index: VEGF index = (VEGFCSF/VEGFserum) / (albuminCSF/albuminserum). Serum VEGF values were used in place of missing plasma VEGF values. This resulted in a lower estimation of the VEGF index, because serum levels of VEGF reflect plasma levels of VEGF plus VEGF released from platelets during clotting.

Immunohistochemical staining of brain specimens. Sections (7 μm) embedded in paraffin were prepared from brain specimens obtained by autopsy. Polyclonal rabbit anti-VEGF and anti–VEGF receptor-1 (Flt-1) antibodies and mouse anti–VEGF receptor-2 (Flk-1/KDR) antibody were used at 2 μg/mL in PBS 2% human serum albumin (Santa Cruz Biotechnology [sc152, sc316, and sc6251]). After deparaffinization and rehydration, the sections were incubated with the secondary antibody (Dako Envision; DAKO) for 1 h at room temperature. Finally, the sections were developed in diaminobenzidine and hydrogen peroxide solution and were counterstained with Mayer's hematoxylin. Polyclonal rabbit anti-factor VIII (A082; DAKO) or omission of the primary antibody served as negative controls. Immunoreactivity was assessed independently by 2 of the authors (M.v.d.F. and G.J.H.v.). Differences were re-examined and discussed to reach consensus. The scoring of the immunoreactivity was confirmed by the pathologist (P.G.J.N.).

Statistical analysis. Data are presented as median (range). Differences between groups were analyzed by the Mann-Whitney U test. Proportions between different groups were compared by Fisher’s exact test.

Results

Patients and controls. During the study period, 37 bacterial meningitis patients (21 male and 16 female) fulfilled the study criteria. The median age of the patients was 19 years (range, 0.5–70). CSF characteristics were as follows: WBC count, 1300 cells/μL (26–25,000); CSF protein, 2.04 g/L (0.23–10); CSF: serum albumin ratio, 31 (3.0–169); and CSF: serum glucose ratio, 0.32 (0.01–0.88). The 51 control subjects (37 male and 14 female) had a median age of 29 years (2 months–78 years). CSF characteristics of the 16 viral meningitis patients were as follows: WBC count, 112 cells/μL (39–540); CSF protein, 0.71 g/L (0.4–1.12); and CSF: serum glucose ratio, 0.62 (0.29–0.91). CSF characteristics of all other control subjects were normal.

VEGF levels. CSF concentrations of VEGF were detectable in 11 (30%) of the 37 bacterial meningitis patients (range <25–633 pg/mL) but in none of the patients with viral meningitis or other control patients (P < .001; figure 1). The calculated VEGF index was 6.2 (0.6–42), a finding that suggests intrathecal production of VEGF. Plasma VEGF levels were

Figure 1. Distribution of vascular endothelial growth factor (VEGF) levels in cerebrospinal fluid (CSF) at admission of 37 patients with bacterial meningitis, 16 patients with viral meningitis, and 35 control patients. The dashed line indicates the detection limit of the assay at 25 pg/mL.
slightly lower in patients than in controls (47 pg/mL [5–570] vs. 74 [8–376]; \( P = .19 \)). Patients with and without detectable VEGF_{CSF} were compared to evaluate whether VEGF_{CSF} levels were related to clinical symptoms, CSF characteristics, causative organism, or outcome. Patients with detectable VEGF_{CSF} presented more frequently with altered mental status (confusion or lethargy in 82% vs. 54%; \( P = .15 \)) and seizures (27% vs. 8%; \( P = .14 \)). No differences were noticed in other symptoms, including nuchal rigidity and nausea or vomiting. Patients with detectable VEGF_{CSF} showed higher CSF WBC counts (2446 cells/\mu L [334–25,000] vs. 960 cells/\mu L [26–15,000]; \( P = .08 \)), higher CSF protein levels (2.5 g/L [1.3–10] vs. 2.0 g/L [0.2–8.7]; \( P = .38 \)), and higher CSF:serum albumin ratios (74 [18–144] vs. 26 [3–169]; \( P = .12 \)). Detectable VEGF_{CSF} levels were seen in 8 (73%) of 11 patients with severe BBB disruption, arbitrarily defined as a CSF:serum albumin ratio >30, versus 12 (46%) of 26 patients with moderate or no BBB disruption (\( P = .13 \)). No significant differences in causative organisms were found between patients with and without detectable VEGF_{CSF}. Streptococcus pneumoniae was isolated in 27% versus 31%, Neisseria meningitidis in 45% versus 46%, S. suis in 0% versus 4%, Listeria monocytogenes in 0% versus 4%, and no isolate in 27% versus 16%, respectively. Death occurred only in patients with elevated VEGF_{CSF} levels (18% vs. 0%; \( P = .83 \)). The 1-month outcome otherwise did not differ significantly in patients with and without VEGF_{CSF}: complete recovery in 64% versus 73% (\( P = .70 \)), hearing loss in 9% versus 8%, impaired cognition in 0% versus 12% (\( P = .55 \)), and focal deficits in 9% versus 8%.

**Immunohistochemistry.** Immunohistochemical analysis of all brain specimens obtained by autopsy showed VEGF immunoreactivity in neutrophils and monocytes of the inflammatory infiltrates in bacterial meningitis patients. VEGF immunoreactivity in ependymal cells, choroid plexus epithelium, meninges, leptomeningeal vessel endothelium, and smooth-muscle and some gial cells was found equally in patients and controls (figure 2). Immunoreactivity for the VEGF receptors Flt-1 and Flk-1/KDR was found in endothelium, neurons, choroid plexus epithelium, and ependymal cells in all subjects (only Flk-1/KDR data are shown; see figure 2).

**Discussion**

In this study, we showed that VEGF was present in the CSF of ~30% of bacterial meningitis patients at the time of admission but in none of the patients with viral meningitis or other control patients. This suggests that VEGF may play a role in the pathophysiology of bacterial meningitis.

Previous reports showed that VEGF can induce endothelial changes during bacterial meningitis, including increased vesicle transport and separation of endothelial intercellular tight junctions. Exposure of normal rat brain to VEGF results in BBB disruption, and VEGF is implicated in the formation of cerebral edema related to brain tumors, trauma, and ischemia [2, 4]. Additionally, dexamethasone, which is used as adjunctive therapy in bacterial meningitis, suppresses tumor-associated brain edema by a mechanism involving down-regulation of VEGF expression [5, 6].

In our study, patients with VEGF_{CSF} more often experienced confusion, lethargy, and seizures and had more severe BBB disruption. Death was observed only in patients with elevated VEGF_{CSF}; however, a statistically significant relation between clinical outcome and VEGF_{CSF} was not present.

The finding that VEGF could not be detected in the CSF of 70% of the bacterial meningitis patients may be explained in several ways. First, migration of neutrophils to the CSF coincides with degranulation of the specific granules that contain VEGF [7, 8]. Locally secreted VEGF may induce endothelial permeability via the luminal receptors of endothelial cells. Notably, intravascular blocking of VEGF reduced brain edema in a mouse model of transient ischemia [2]. Local VEGF secretion by migrating neutrophils may not be reflected in circulating VEGF levels, because we found no differences in plasma VEGF. Disruption of the BBB may be further aggravated by VEGF secreted in the CSF via the abluminal receptors of endothelial cells. Second, the timing of sampling may affect VEGF_{CSF} detection, since the in vivo half-life of VEGF_{CSF} is unknown. Finally, BBB disruption during bacterial meningitis involves a complex interaction between neutrophils and mediators, such as interleukin-1, platelet-activating factor, nitric oxide, and matrix metalloproteinases, which might induce BBB permeability independent of VEGF [9].

Exceptionally high concentrations of VEGF are found in the CSF in carcinomatous meningitis (median, 7000 pg/mL), as a result of abundant VEGF production by tumor cells [10]. The levels =633 pg/mL that we measured in the bacterial meningitis patients appear to be biologically relevant, since VEGF is already active at concentrations of 40 pg/mL in vitro [11].

In the brain specimens obtained by autopsy from bacterial meningitis patients, VEGF immunostaining was clearly present in the meningeal inflammatory infiltrate, indicating that neutrophils are the main source of VEGF in the CSF. This finding is further substantiated by the fact that patients with detectable VEGF_{CSF} had higher CSF WBC counts, approaching statistical significance (\( P = .08 \)). Inflammatory stimuli such as tumor necrosis factor-α and pneumococcal cell wall may induce VEGF secretion by neutrophils and monocytes in the CSF [7, 12]. Whether ependymal cells and choroid epithelial cells contribute to VEGF_{CSF} levels remains unclear. No differences between patients and controls in VEGF distribution pattern were noted in these cells. Unexpectedly, we found VEGF immunoreactivity in endothelium and smooth-muscle cells, specifically in the leptomeninges, as was described in a rat model of transient ischemia [13]. This suggests that disease-related hypoxia preceding death may have influenced VEGF localization in the brains of both patients and controls.

We detected the VEGF receptors Flt-1 and Flk-1/KDR in
Figure 2. Representative localization of vascular endothelial growth factor (VEGF) and VEGF receptor–2 (fetal liver kinase–1 [Flk-1]/kinase insert domain-containing receptor [KDR]) in brain specimens of bacterial meningitis patients. A, VEGF immunoreactivity was found in the leptomeningeal infiltrate and in the leptomeningeal vessel walls (arrows). B, Flk-1/KDR was not detected in the inflammatory infiltrate or in vessel walls (arrows). C, Leptomeningeal inflammatory infiltrate at greater magnification shows that VEGF was found in neutrophils (arrows) and monocytes (open arrow) but not in lymphocytes (arrowheads). D, No Flk-1/KDR immunoreactivity was observed in the inflammatory infiltrate. E and F, VEGF and Flk-1/KDR were detected both in ependymal cells and in choroid plexus epithelium. G and H, VEGF was absent from neurons, but neurons were Flk-1/KDR positive. Scale bars correspond to 50 mm (original magnification, ×100 in A and B; ×1000 in C and D; and ×400 in E–H).
neurons of brain specimens of both patients and controls. This may indicate that VEGF has direct effects on neurons, a property that may be independent of the vascular permeability effect. Indeed, recently VEGF was found to have direct neuroprotective effects in vitro ischemia [14]. Furthermore, topical application of VEGF on the cerebral cortex induced a reduction of infarct size in a rat model of transient cerebral ischemia [15].

In conclusion, we have shown that VEGF can be detected in the CSF of patients with bacterial meningitis, in whom the inflammatory infiltrate appears to be the main source of secretion. Consistent with its role in edema in brain tumors, trauma, and infarction, VEGF may be a novel mediator of BBB disruption in bacterial meningitis. Further research is required to elucidate the exact role of VEGF in this disease.

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