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BRIEF REPORT

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Soluble triggering receptor expressed on myeloid cells 1: a biomarker for bacterial meningitis

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Abstract *Objective:* To evaluate whether soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) in CSF can serve as a biomarker for the presence of bacterial meningitis and outcome in patients with this disease. *Design:* Retrospective study of diagnostic accuracy. *Setting and patients:* CSF was collected from 92 adults with community-acquired bacterial meningitis who participated in the prospective Dutch Meningitis Cohort Study; 8 patients with viral meningitis and 9 healthy control subjects. *Results:* CSF sTREM-1 levels were higher in patients with bacterial meningitis (median 82 pg/ml, range 0–988) than in those with viral meningitis (0 pg/ml, 0–48) and controls (0 pg/ml, 0–36). The diagnostic accuracy of sTREM-1 in discriminating between patients with and without bacterial meningitis, expressed as the area under the receiver operating characteristic curve, was 0.82. At a cutoff level of 20 pg/ml the sensitivity was 0.73 and specificity 0.77. In patients with bacterial meningitis CSF sTREM-1 levels were associated with mortality (survivors, median 73 pg/ml, range 0–449 pg/ml; nonsurvivors, 151 pg/ml, 0–988). *Conclusions:* Measuring sTREM-1 in CSF may be a valuable new additional approach to accurately diagnose bacterial meningitis and identify patients at high risk for adverse outcome. Therefore a prospective study of sTREM-1 as a biomarker in bacterial meningitis is needed.

Keywords Soluble triggering receptor expressed on myeloid cells 1 · Bacterial meningitis · Viral meningitis · Cerebrospinal fluid · Outcome

Introduction

Bacterial meningitis is a life-threatening disease [1]. In adults *Streptococcus pneumoniae* and *Neisseria meningitidis* are the predominant causes, with an overall case fatality rate of approx. 30% and 10%, respectively. Early use of dexamethasone and antibiotics improves prognosis, and therefore early diagnosis is vital [2]. Triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently discovered cell surface molecule whose expression on phagocytes is upregulated by exposure to bacteria or fungi [3]. A soluble form of TREM-1 (sTREM-1) can be

measured in various body fluids [4, 5]. In patients receiving mechanical ventilation sTREM-1 levels in bronchoalveolar fluid have been shown to be a good indicator of infectious pneumonia [5, 6]. This study evaluated sTREM-1 levels in cerebrospinal fluid (CSF) of adults with meningitis and explored its diagnostic accuracy in the differentiation between bacterial and viral meningitis. In addition, we studied whether sTREM-1 concentrations in CSF from patients with bacterial meningitis are associated with mortality. Parts of these data have been presented at an international meeting [7].

Materials and methods

Levels of sTREM-1 in CSF from patients with bacterial meningitis ($n = 92$) included in the Dutch Meningitis Cohort were compared with those from patients with viral meningitis ($n = 8$) and controls ($n = 9$). Demographic characteristics (age and sex) were similar between patients with bacterial meningitis, viral meningitis, and controls; among those with bacterial meningitis the patients with pneumococcal meningitis were older than those with meningococcal meningitis. The Dutch Meningitis Cohort prospectively included 696 episodes of community-acquired bacterial meningitis, confirmed by culture of CSF, in patients aged over 16 years in The Netherlands between October 1998 and April 2002. Inclusion and exclusion criteria are described more extensively elsewhere [1]. CSF samples were centrifuged and routinely collected in 92 of 696 episodes (13%); 56 of 92 (61%) with pneumococcal meningitis, 25 (27%) with meningococcal meningitis and 11 (12%) with other bacterial pathogens (*Listeria monocytogenes* in 3, *Staphylococcus aureus* in 2, *Haemophilus influenzae* in 2, and *Escherichia coli*, *Klebsiella pneumoniae*, streptococcus groups A and C both in 1). Although clinical data and CSF samples were collected prospectively, the present study on diagnostic accuracy was designed retrospectively. On receipt CSF samples were stored at 70°C. Patients with viral meningitis and controls were selected from archives of the Laboratories of the Departments of Virology and Neurogenetics (2003–2005), both located at the Amsterdam Medical Center. Patients with viral meningitis had viral DNA in CSF detected by polymerase chain reaction ($n = 8$; herpes simplex virus in 5, varicella zoster virus in 2, Epstein-Barr virus in 1). The control group ($n = 9$) consisted of healthy subjects who underwent lumbar puncture to exclude the diagnosis of subarachnoid hemorrhage (blood degradation products were negative and CSF white cell count fewer than 5 cells/mm^3 in all). Levels of sTREM-1 were determined by enzyme-linked immunosorbent assay, as described previously [6]. In short, 96-well plates precoated with rabbit anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark) were coated overnight with 400 ng mouse anti-human TREM-1 antibody (R&D Systems, Minneapolis, Minn., USA). Calibrator (R&D Systems), controls and samples diluted as appropriate were added and incubated for 2 h. Next 40 ng of biotinylated goat anti-human TREM-1 antibody (R&D Systems) was added and incubated for another 2 h. Streptavidin poly-horseradish peroxidase was added for 30 min. Finally, sodium acetate buffer (pH 5.5) containing 100 μg/ml tetramethylbenzidine and 0.003% H_2O_2 was added, and the color reaction was stopped by 2 M H_2SO_4. The detection limit of the assay was 20 pg/ml, and all measurements were made in duplicate.

Descriptive results of continuous data are expressed as medians and range. To identify differences between groups the Mann–Whitney *U*, Kruskal–Wallis, χ^2, or Fisher's exact statistics were used. Strength of relationships between sTREM-1 level and clinical or biological features was assessed by Spearman's correlation test. A receiver operating characteristic curve (ROC) was constructed to illustrate various cutoff CSF levels of sTREM-1 in differentiating between the presence and absence of bacterial meningitis, and diagnostic accuracy was quantified by 95% confidence intervals (95% CI). Analyses were carried out with SPSS, version 12.01.

Results

CSF white cell count was performed in 85 of 92 patients with bacterial meningitis (92%) and in all patients with viral meningitis and controls; in one additional patient the CSF specimen contained too many white cells to perform exact counting. CSF white cell count was higher in patients with bacterial meningitis (median 1,917 cells/mm^3, range 1–127,710) than in those with viral meningitis (166 cells/mm^3, 24–471; $p = 0.002$). Patients with viral meningitis had higher CSF white cell counts than controls ($p < 0.001$). Patients with bacterial meningitis had higher CSF protein levels than those with viral meningitis (median 4.7 g/l, 0.3–18.8, vs. 1.0 g/l, 0.4–8.1 g/l; $p = 0.008$), and a lower ratio of CSF glucose to blood glucose (median 0.07, range 0–0.89, vs. 0.59, 0.48–0.78; $p < 0.001$). Pneumonia was present on admission in 16 patients (17%) and 14 patients (15%) were considered to be immunocompromised due to the use of immunosuppressive drugs, diabetes mellitus, alcoholism or asplenia. Sixteen of 90 patients (18%) received steroids, and adjunctive dexamethasone was initiated before the first dose of antibiotics in five patients (6%). Patients who died had a higher severity of disease, reflected by a lower score on the Glasgow Coma Scale ($p = 0.008$), than those surviving bacterial meningitis.

In all 92 CSF samples sTREM-1 levels were determined in duplicate; variability between the two measurements was low (7.6%). Levels of sTREM-1 in CSF were higher in patients with bacterial meningitis than

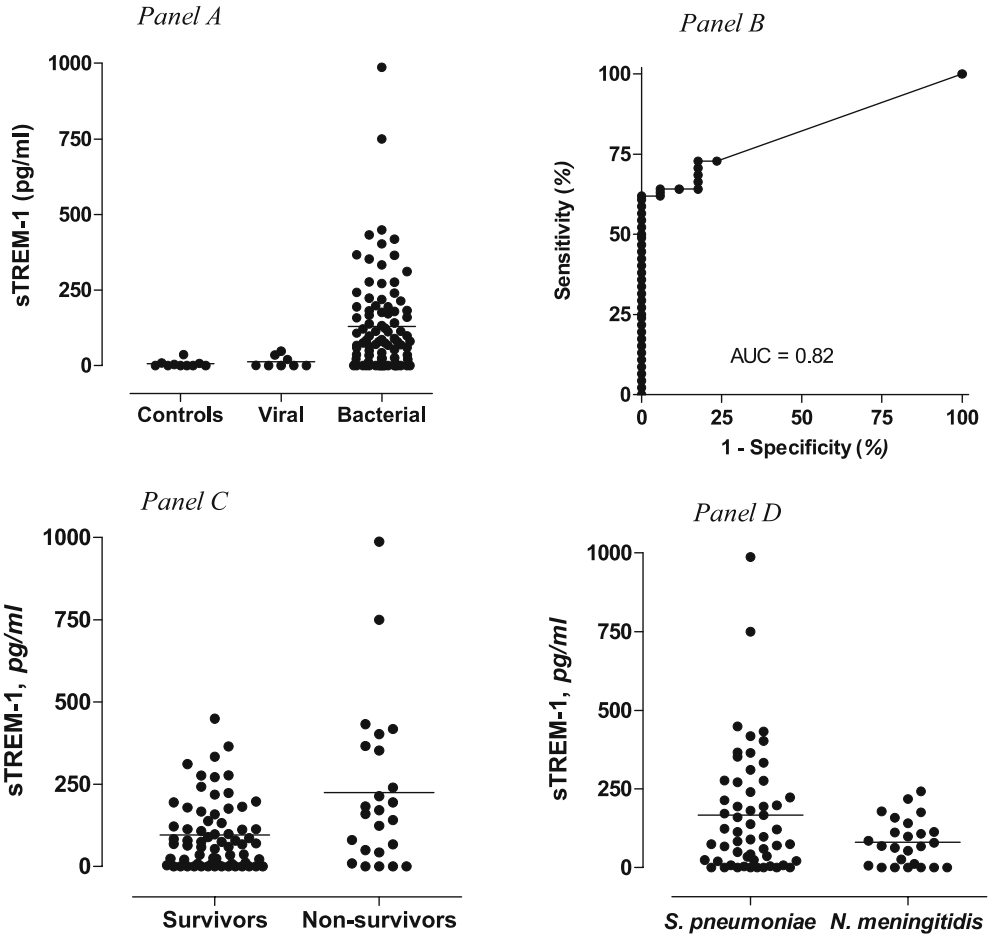

Fig. 1 Cerebrospinal fluid levels of soluble triggering receptor expressed on myeloid cells-1. *Panel A* CSF sTREM-1 levels in patients with bacterial meningitis ($n = 92$), patients with viral meningitis ($n = 8$), and controls ($n = 9$). *Panel B* Receiver-operating characteristics curve for various cutoff CSF levels of sTREM-1 in differentiating between bacterial meningitis ($n = 92$) and absence of bacterial meningitis ($n = 17$); the area under the receiver-operatingcharacteristics curve was 0.82 (95% CI 0.74–0.90). *Panel C* CSF sTREM-1 levels in survivors ($n = 68$) and nonsurvivors ($n = 24$) of bacterial meningitis. *Panel D* CSF sTREM-1 levels in patients with meningitis caused by *Streptococcus pneumoniae* ($n = 56$) and *Neisseria meningitidis* ($n = 25$)

in those with viral meningitis (median 82 pg/ml, range 0–988, vs. 0 pg/ml, 0–48 pg/ml; $p = 0.006$; Fig. 1) and controls (0 pg/ml, range 0–36; $p = 0.001$). Patients with viral meningitis and controls had similar CSF sTREM-1 levels.

In the next step we explored the diagnostic accuracy of sTREM-1 in CSF discriminating between patients with and without bacterial meningitis (including patients with viral meningitis and controls). In this analysis the area under the ROC curve (AUC) was 0.82 (95% CI 0.74–0.90; $p < 0.001$; Fig. 1). At a cutoff level of 20 pg/ml sTREM-1 yielded a sensitivity of 0.73 (95% CI 0.65–0.80) and specificity of 0.77 (0.57–0.89). The positive predictive value was 0.94 (95% CI 0.88–0.98), negative predictive value 0.34 (0.23–0.48), and positive likelihood ratio 3.1 (1.5–6.4). To assess the additional value of sTREM-1 determination we identified all patients with at least one individual CSF finding considered predictive of bacterial meningitis by the criteria of Spanos et al. [8] (glucose level < 1.9 mmol/l, ratio of CSF glucose to blood glucose < 0.23, protein level > 2.20 g/l, white cell count > 2000/mm^3 or CSF neutrophil count > 1180/mm^3). In 88 of 92 patients with bacterial meningitis (96%) additional CSF characteristics were determined; in 77 of these 88 patients (88%) bacterial meningitis was identified by the Spanos criteria. Of the remaining 11 of a total of 88 patients (13%), 3 patients (27%) had CSF sTREM-1 levels above the cutoff level of 20 pg/ml.

Concentrations of sTREM-1 in CSF from patients with bacterial meningitis were associated with mortality (survivors, median 73 pg/ml, range 0–449 pg/ml; nonsurvivors, 151, 0–988; $p = 0.02$; Fig. 1). CSF sTREM-1 level did not differentiate between pneumococcal meningitis and meningococcal meningitis (median 108 pg/ml, range 0–988, vs. 73, 0–243; $p = 0.07$; Fig. 1). Clinical features associated with sTREM-1 levels were erythrocyte sedimentation rate (Spearman's test 0.37, $p = 0.001$), CSF glucose level (–0.34, $p = 0.002$), and CSF protein level (0.47, $p < 0.001$). There was no correlation between sTREM-1 levels, antibiotic use before admission, CSF white cell count, C-reactive protein, or any other clinical or biological feature.

Discussion

Our findings show that sTREM-1 in CSF may be helpful as an additional diagnostic tool in the discrimination between patients with bacterial and viral meningitis. In

addition, CSF sTREM-1 levels have prognostic value in patients with bacterial meningitis. Measuring sTREM-1 in CSF may be a valuable new approach to accurately diagnose bacterial meningitis and identify patients at high risk for adverse outcome. Therefore a prospective study on sTREM-1 as biomarker in bacterial meningitis is needed. Clinical features of patients with bacterial and viral meningitis may be remarkably similar and CSF findings inconclusive [9]. In a recent retrospective cohort that included 144 adults with bacterial or viral meningitis Brivet et al. [10] found that the presence of at least one sign of severity at referral (altered consciousness, seizures, focal neurological findings, and shock) and a CSF absolute neutrophil count higher than 1,000/mm^3 were independent predictors for the presence of bacterial meningitis. Other previous prognostic models included CSF parameters alone or in combination with clinical characteristics and yielded a high diagnostic accuracy in identifying patients with bacterial meningitis [11, 12]. We showed that sTREM-1 determination may have additional value to routine CSF parameters. Whether determination of sTREM-1 in CSF has additional value to other routine CSF parameters, serum procalcitonin, and prognostic models should be an objective of further research. Our study was retrospective and included only a limited number of patients and controls, and therefore care must be taken before drawing any firm conclusions. Although the diagnostic accuracy of CSF sTREM-1 levels was high, there was a considerable overlap in CSF sTREM-1 values between patients with bacterial and viral meningitis. This may have been due to a relatively low sensitivity of the assay. Other important limitations are the uncertainty of the exact period between lumbar puncture and storage of the CSF samples, which may have affected sTREM-1 levels.

The sTREM-1 levels in CSF were higher among patients with bacterial meningitis with lethal outcome than among patients who survived. Levels of sTREM-1 were related to low CSF glucose and high CSF protein levels but not to CSF white cell count nor to causative organism. One biological explanation of this might be excessive bacterial growth in the subarachnoid space, triggering high sTREM-1 levels, with a relatively low white-cell response in the CSF. Supportive to this notion are studies of animals with pneumococcal meningitis showing a relationship between a large bacterial CSF load, lack of response of CSF leukocytes, and intracranial complications [13]. As influx of leukocytes into the subarachnoid space is a hallmark of bacterial meningitis, sTREM-1 is probably due to local expression. However, as sTREM-1 levels in serum were not determined, sTREM-1 originating from the circulation may have entered the CSF by diffusion after disruption of the blood-brain barrier. Moreover, sTREM-1 levels were not correlated with CSF leukocyte count, but the correlation with the erythrocyte sedimentation rate was highly significant. In a small proportion of patients there were no individual CSF characteristcs predictive of bacterial meningitis and sTREM-1 levels were below the cutoff level of 20 pg/ml. A possible explanation is that these patients underwent lumbar puncture early in the course of disease, which is in accord with the finding that low levels of sTREM-1 are related to favorable outcome.

In conclusion, determination of sTREM-1 levels in CSF may be of diagnostic and prognostic value in bacterial meningitis. It may have additional value to traditional CSF markers of bacterial meningitis. Prospective cohort studies including patients with suspected bacterial meningitis, preferably collecting CSF and serum samples at multiple time points, are needed to substantiate our findings.

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