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Macrophage migration inhibitory factor as a novel cerebrospinal fluid marker for neurosyphilis among HIV-negative patients

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ABSTRACT

Background: Neurosyphilis (NS) is difficult to diagnose, especially in syphilis patients with negative cerebrospinal fluid (CSF) rapid plasma reagin (RPR) or Venereal Disease Research Laboratory (VDRL) tests.
Methods: We conducted a cross-sectional study and an analysis of macrophage migration inhibitory factor (MIF) in syphilitic patients to identify a novel marker for the diagnosis of NS, with a focus on probable NS (NS with negative VDRL/RPR tests). For this purpose, CSF and serum MIF concentrations were determined in 43 NS and 43 syphilis/non-NS (N-NS) patients at the Zhongshan Hospital of the Medical College of Xiamen University from July 2014 to June 2015. Sixty-three blood donors were used as healthy controls.
Results: NS patients had higher CSF (median [IQR]: 8.77 ng/ml [4.76–19.13]) and serum (52.58 ng/ml [28.31–95.94]) MIF concentrations than N-NS patients did (4.08 [2.21–9.68] and 34.30 [19.77–59.75], respectively). Using a cut-off point of 6.63 ng/ml, CSF MIF had a sensitivity of 74.42% and a specificity of 67.74% for the diagnosis of NS. The sensitivity was higher than that of CSF RPR (39.53%) and increased protein (48.84%) tests and similar to that of CSF pleocytosis (67.44%). Additionally, the sensitivity of CSF MIF, which was 92.31% for the diagnosis of probable NS, was higher than that of CSF pleocytosis (65.38%) and increased protein (53.85%) tests. By integrating all CSF parameters (pleocytosis, increased protein and MIF), the sensitivity would be improved to 100% by parallel testing, which would avoid missed diagnoses. Moreover, the specificity would be improved to 100% by the serial testing algorithm, which would again avoid misdiagnosis.
Conclusions: CSF MIF concentrations can be used as a novel CSF marker to establish or exclude a diagnosis of NS.

1. Introduction

Approximately 70% of early-stage syphilis patients have central nervous system (CNS) invasion [1]. Invasion of the cerebrospinal fluid (CSF) by *Treponema pallidum* may be followed by spontaneous clearance, without development of an inflammatory response; by development of a transient inflammatory response; or by development of a persistent inflammatory response. The third outcome defines asymptomatic neurosyphilis (NS), which is the precursor of symptomatic NS [2]. The clinical spectrum of NS is very broad and nonspecific, which not only creates diagnostic problems but also results in poor therapeutic decisions. Inflammatory CSF parameter analysis plays an essential role in the diagnosis of NS, which includes reactive Venereal Disease Research Laboratory (VDRL), pleocytosis and increased protein tests [3]. The CSF VDRL test, which may be replaced by the rapid plasma reagin (RPR) test in certain areas [4], is the preferred laboratory test for NS diagnosis, with a high specificity (99.80%) and a low sensitivity (range 30.00–70.00%) [5]. Typically, syphilitic patients with a reactive CSF VDRL test can be confirmed as having NS, whereas a negative CSF VDRL test cannot exclude the possibility of NS. CSF pleocytosis or increased CSF protein tests have been typically used for auxiliary NS diagnosis when syphilitic patients present a negative CSF VDRL test [3], but CSF pleocytosis is only observed in 40.00–59.10% of NS patients. Similarly, increased protein tests are reactive in only 41.20–56.40% of NS patients [6,7]. To date, no gold-standard test has been developed for the diagnosis of NS, especially among syphilitic patients with a negative CSF VDRL test; researchers are thus seeking new predictors for the early recognition of NS to avoid missed diagnoses or misdiagnosis.

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Macrophage migration inhibitory factor (MIF) is a unique cytokine and a critical mediator of host defences that plays a role in chronic inflammation [8]. MIF is a crucial indicator of CNS infection, with multiple clinical studies noting the utility of MIF as a biomarker for different diseases that have an inflammatory component [9]. However, few research studies have analysed the association between MIF and syphilis. J. Podwinska et al. specifically examined the MIF concentration of syphilitic patients at different stages and found that the serum MIF concentration increases during primary syphilis, reaching the highest value during secondary syphilis (with the exception of asymptomatic NS), but no data on CSF MIF exist [10]. Here, we conducted a cross-sectional study and an analysis of MIF in NS patients, including serum MIF and CSF MIF data, to identify a novel marker for the diagnosis of NS, with a focus on probable NS.

2. Materials and methods

2.1. Study design and patients

This cross-sectional study was conducted at the Zhongshan Hospital of the Medical College of Xiamen University from July 2014 to June 2015. A total of 556 patients were clinically diagnosed with syphilis by combining serodiagnosis and medical history. All patients had a positive serum *T. pallidum* particle agglutination (TPPA) test. The following patients were excluded from the study: returning patients; those without a lumbar puncture; and those with HIV infection, other CNS infections with known causes, concurrent CNS infections, a history of systemic diseases (e.g., autoimmune diseases, malignancy, hypertension, and diabetes mellitus), or dermatological diseases other than syphilis (as these are known to increase the MIF concentration) [11]. In total, 86 HIV-negative syphilis patients with CSF examinations were enrolled in this study (Fig. 1), including 43 NS patients and 43 syphilis/non-NS (N-NS) patients. Additionally, 63 age- and sex-matched blood donors were included as the healthy controls. The patients' demographic and clinical characteristics were obtained from medical records. This study was approved by the Institutional Ethics Committee of the Zhongshan Hospital of the Medical College of Xiamen University, and it was in compliance with national legislation and the Declaration of Helsinki guidelines. Written informed consent was also solicited prior to collection of the samples.

2.2. Diagnostic criteria

The diagnoses of syphilis and NS were based on the European guidelines as well as the US Centers for Disease Control guidelines [12,13]. Because the diagnostic criteria for NS are still debated, the NS cases were further subdivided into confirmed NS and probable NS. Confirmed NS was defined as syphilis at any stage with a reactive RPR test for CSF. Probable NS was defined as syphilis of any stage with a negative CSF RPR test and both of the following: (1) a CSF white blood cell count > 10 cells/μl and/or a CSF protein concentration >500 mg/l and (2) clinical symptoms or signs consistent with NS without other known causes for these clinical abnormalities. NS is categorized as early or late stage: early NS is a stage with or without symptoms that includes asymptomatic NS, syphilitic meningitis and meningovascular NS, and late NS primarily affects the CNS parenchyma, with associated clinical syndromes including general paresis and tabes dorsalis [14]. N-NS was defined as syphilis at any stage, excluding NS.

2.3. Principle of the MIF assay

CSF and blood samples were obtained at the time of diagnosis and were transported directly to the Center Clinical Laboratory, where the samples were centrifuged. The supernatants were then collected and stored at −80 °C until being assayed. MIF in the CSF and serum was measured using human ELISA kits (Boster Biological Engineering Co., Ltd.) according to the manufacturer's instructions.

2.4. Syphilitic serologic tests

The syphilitic serologic tests for each sample were performed using the RPR (InTec) and TPPA (Fujirebio) assays according to the manufacturers' instructions and as previously reported [15,16].

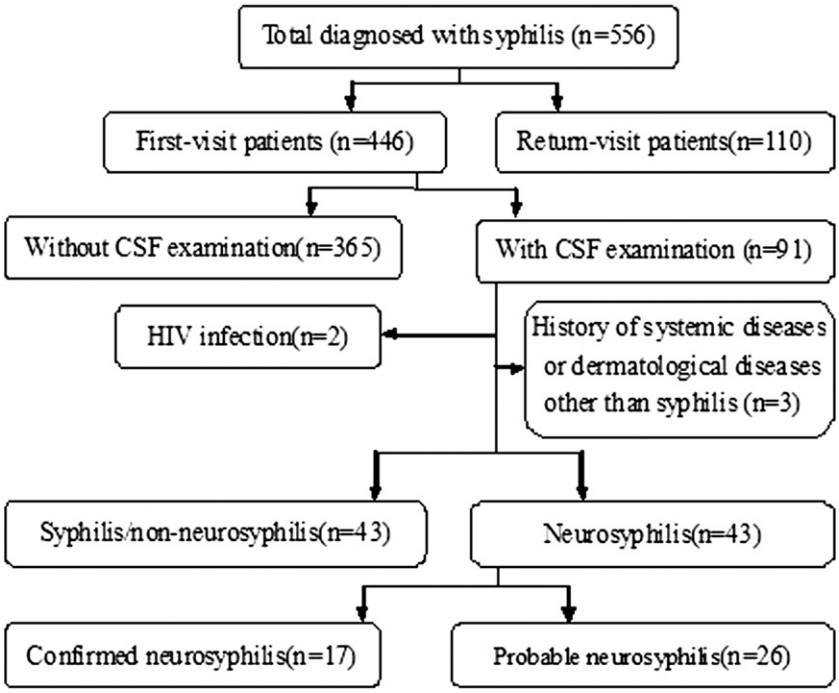

Fig. 1. The flow of participants from enrolment.