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 67.44% 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 (75.38%) 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.

Keywords: Macrophage migration inhibitory factor; *Treponema pallidum*; Neurosyphilis;

Diagnosis; Marker

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.

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 greater than 10 cells/ μ l 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].

2.5 Examination of CSF protein and white blood cell count

Approximately 2 ml of each CSF sample was collected in a plain sterile tube and analysed within 1 hour to measure the protein content using a Roche-Hitachi Modular P800 analyser (Roche Diagnostics), and the CSF white blood cell count was determined using an automatic blood cell XE5000 analyser (Sysmex International Reagents Co.) [17].

2.6 Data analysis

Statistical analyses were performed using SPSS ver 13.0 software. Non-normal data are described using the median and interquartile range (IQR), and categorical variables are

described using numbers and percentages. Fisher's exact test was used to analyse categorical variables, and the Mann-Whitney test was used for continuous variables with a skewed distribution. Additionally, descriptive data are summarized using the mean and standard deviation when they are approximately normally distributed. The differences between experimental groups were analysed using one-way ANOVA followed by the Bonferroni test for selected pairs. A $P \leq 0.05$ was considered significant. The sensitivity and specificity of CSF and serum parameters were calculated using a 2-by-2 table.

3. Results

3.1 Characteristics of the study participants

The clinical information of the study participants is shown in Table 1. In this study, the NS, N-NS, and healthy control groups exhibited no significant differences in age ($F=0.31$, $P=NS$) or sex ($\chi^2=1.25$, $P=NS$). Using an age of ≥ 50 y as the immunologic cut-off for older age in accordance with previous reports [18], we found that the proportions of older participants in the three groups were not different ($\chi^2=4.85$, $P=NS$). The NS patients had a significantly higher rate of positive serum RPR tests than the N-NS patients did ($\chi^2=17.98$, $P=0.00$), whereas the rate of positive serum TPPA tests in both the NS and N-NS groups was 100%. The rates of positive CSF RPR tests and CSF TPPA tests were 39.53% and 67.44%, respectively, in the NS group, and the rates of positive CSF RPR tests and CSF TPPA tests were both negative in N-NS patients. Compared to the N-NS group, NS patients had higher rates of CSF pleocytosis ($\chi^2=20.91$, $P=0.09$) and CSF increased protein ($\chi^2=12.14$, $P=NS$). Eleven N-NS patients (11/43, 25.58%) had CSF abnormalities, including 8 patients (8/43,

18.60%) with CSF pleocytosis ($>10^6$ cells/l), 6 patients (6/43, 13.95%) with an increased CSF protein concentration (>500 mg/l), and 3 patients (3/43, 6.98%) with both CSF pleocytosis and increased CSF protein concentrations (data not shown).

3.2 CSF and serum MIF concentrations in the different groups

The serum MIF concentrations in the NS cohort (median 52.58 ng/ml, IQR [28.31-95.94]) were significantly higher than those of N-NS patients (median 34.30 ng/ml, IQR [19.77-59.75]) ($z=-2.73$, $P=0.01$) as well as those of healthy subjects (median 11.20 ng/ml, IQR [7.89-23.69]) ($z=-6.63$, $P=0.00$); in particular, the concentrations of serum MIF in healthy subjects were the lowest among the three groups. In addition, the concentrations of CSF MIF (median 8.77 ng/ml, IQR [4.76-19.13]) in NS patients were higher than those in N-NS patients (median 4.08 ng/ml, IQR [2.21-9.68]) ($z=-3.10$, $P=0.00$) (Table 2). Furthermore, we analysed the MIF concentrations in early and late NS and found that the CSF MIF concentration in late NS was significantly higher than that in early NS ($z=-5.26$, $P=0.00$). In contrast, no significant difference was observed in serum MIF concentrations between early NS and late NS ($z=-1.17$, $P=0.24$) (Table 2). Furthermore, no differences in gender or age were found among the three groups for either the serum MIF or the CSF MIF concentrations (data not shown).

3.3 Diagnostic sensitivity and specificity of MIF for NS screening

To evaluate the use of the CSF MIF concentration as a screening test for NS, the screening accuracy was determined using receiver operating characteristic (ROC) curve

analysis, and the area under the curve for CSF MIF was 0.694 (95% CI, 0.585-0.789). The maximal Youden's index further indicated that 6.63 ng/ml CSF MIF was the best cut-off point to distinguish between NS and N-NS, leading to a sensitivity of 74.42%, a specificity of 67.74%, and a negative predictive value (NPV) of 72.50% for NS screening (Fig. 2A, Table 3). We also analysed the use of the serum MIF concentration for NS screening using ROC curve analysis and determined the area under the curve for serum MIF to be 0.671 (95% CI, 0.561-0.768). The cut-off value for serum MIF was 60.49 ng/ml, which led to a sensitivity of 48.84%, a specificity of 79.07% and an NPV of 60.00% (Fig. 2B, Table 3).

Using clinical diagnosis as the gold standard, we analysed the sensitivity, specificity, positive predictive value (PPV) and NPV of CSF RPR, CSF pleocytosis (>10 cells/ μ L), CSF increased protein (>500 mg/l) and CSF MIF testing as screening tests for NS using 2-by-2 tables (Table 3). The CSF MIF (>6.63 ng/ml) concentration had significantly higher sensitivity than the CSF RPR ($\chi^2=23.58$, $P=0.00$) and CSF increased protein ($\chi^2=13.20$, $P=0.00$) tests did and a similar sensitivity to that of CSF pleocytosis ($\chi^2=1.18$, $P=0.35$). The sensitivities of CSF MIF, CSF pleocytosis, CSF increased protein and CSF RPR testing were specifically 74.42%, 67.44%, 48.84% and 39.53%, respectively. We further divided NS patients into two sub-groups, namely, probable NS and confirmed NS, and analysed the diagnostic efficiency of the above test indexes. The results indicated that the sensitivity of increased CSF MIF was significantly higher than that of CSF increased protein ($\chi^2=36.63$, $P=0.00$) and CSF pleocytosis ($\chi^2=27.60$, $P=0.00$) in the probable NS group and that the sensitivity of CSF MIF (>6.63 ng/ml) was significantly higher than that of CSF increased protein ($\chi^2=8.91$, $P=0.00$) but was not different from that of CSF pleocytosis ($\chi^2=2.91$,

$P=0.12$) in the confirmed NS group. Interestingly, we found that a cut-off value for CSF MIF of >6.63 ng/ml resulted in a high sensitivity (92.31%) and NPV (92.59%) for diagnosing probable NS. Therefore, approximately 7.41% of individuals with a negative CSF MIF result may be misdiagnosed with probable NS. A similar NPV (90.00%) for CSF MIF was shown in the confirmed NS group. Meanwhile, the serum MIF concentration showed poor diagnostic efficiency: the sensitivity values for serum MIF for diagnosing NS, probable NS, and confirmed NS were 48.84%, 46.15%, and 52.94%, respectively, which were similar to those for CSF increased protein (Table 3).

3.4 Parallel and serial testing algorithms for CSF parameters for NS screening

To improve the diagnostic efficiency of NS screening, we combined the CSF pleocytosis and CSF increased protein tests in a parallel testing format that utilized CSF pleocytosis, increased CSF protein or both. The algorithm produced a sensitivity of 83.72%, a specificity of 74.42%, and an NPV of 82.05% for NS screening; a sensitivity of 80.77%, a specificity of 74.42%, and an NPV of 86.49% for probable NS; and a sensitivity of 94.12%, a specificity of 55.07%, and an NPV of 97.44% for confirmed NS. When we added the increased CSF MIF results to the parallel testing format, the sensitivity values of the algorithm for screening for NS, probable NS, and confirmed NS were 97.67%, 100%, and 100%, respectively, and the NPVs were 95.24%, 100%, and 100%, respectively (Table 4).

We also generated a serial testing algorithm that utilized both CSF pleocytosis and CSF increased protein results to identify NS. This algorithm produced a specificity of 93.02% and a PPV of 82.35% for NS screening, a specificity of 93.02% and a PPV of 57.14% for

probable NS, and a specificity of 89.86% and a PPV of 58.82% for confirmed NS. When we added the increased CSF MIF results to the algorithm, the specificities of the algorithm for screening for NS, probable NS, and confirmed NS were 100%, 100%, and 94.20%, respectively, and the PPVs were 100%, 100%, and 69.23%, respectively (Table 4).

4. Discussion

NS, which is known as ‘the great mimicker’, has no typical symptoms or signs and can mimic any other CNS disease. The accurate diagnosis of NS is difficult because the common definition of NS depends on a combination of CSF parameters [6,19]. According to the most rigorous standard, a reactive CSF VDRL or RPR test establishes a diagnosis of confirmed NS [4, 20] and is considered specific for NS but is not sensitive. In our study, 39.53% of NS patients had reactive CSF RPR tests, which is lower than the percentage previously reported in the literature (50%) [5]. In patients with negative CSF RPR tests, CSF inflammatory parameters, including CSF pleocytosis and CSF increased protein, were considered diagnostic indicators of probable NS. In this study, CSF pleocytosis (white blood cell count $>10/\mu\text{L}$) and CSF increased protein ($>500\text{ mg/l}$) occurred in 67.44% and 48.84% of patients, respectively, and both were positive in 14 cases, similar to the results of previous studies [6,7]. CSF pleocytosis and CSF increased protein were also positive in N-NS patients, with rates of 18.60% and 13.95%, respectively. The diagnosis of NS is still a challenge for clinicians; in particular, the diagnosis of probable NS patients with negative CSF RPR tests can be especially difficult. Moreover, the CSF pleocytosis and CSF increased protein tests are

not specific and have low sensitivity.

MIF is an important multifunctional cytokine that is synthesized by several cell types in the brain [21]. This cytokine has a direct chemokine-like function and promotes cell recruitment, with broad, upstream actions in the inflammatory cascade. Once released, MIF exerts critical autocrine and paracrine activating effects, including induction of the inflammatory cytokines nitric oxide, cyclooxygenase-2, and prostaglandin oestrogen-2 [22]. Multiple clinical studies have noted the utility of MIF as a biomarker for different diseases that have an inflammatory component; these include systemic infections and sepsis, autoimmune diseases, cancer, and metabolic disorders such as type 2 diabetes and obesity [9]. MIF also plays putative roles in several CNS inflammatory conditions. In the present study, the concentration of serum MIF was highest in the NS group and lowest in the healthy controls. The serum MIF concentration in the healthy controls was 11.20 ng/ml (IQR [7.89-23.69]), which is similar to what was found in previous studies [23]. The CSF MIF concentration of NS patients was higher than that of N-NS patients. With an optimal cut-off value of 6.63 ng/ml, the CSF MIF concentration produced a sensitivity of 74.42% and a specificity of 67.44% for the diagnosis of NS. The sensitivity was higher than that of positive CSF RPR and increased protein tests and similar to that of CSF pleocytosis. For probable NS, the results showed that the sensitivity of CSF MIF (92.31%) was higher than that of CSF pleocytosis and CSF increased protein, and it also had a high NPV, or 92.59%. Thus, 92.59% of patients excluded by this screening test will be correctly identified as having N-NS. Surprisingly, the CSF MIF concentration was significantly higher in late NS, whereas the

serum MIF concentration showed no significant difference between early and late NS. This result may have occurred because CSF MIF is synthesized by and released from neurons as well as astrocytes [24] and because late NS affects the CNS parenchyma and leads to MIF release from injured neurons.

CSF analysis plays an essential role in the assessment of NS, and it is important to integrate all of the CSF parameters (including CSF pleocytosis and CSF increased protein) to accurately diagnose probable NS. In our study, the sensitivity of parallel testing (CSF pleocytosis and CSF increased protein) was 80.77%, and the sensitivity increased to 100% when the CSF MIF concentration was added to the parallel testing; all of the probable NS cases would be screened out at this threshold. Therefore, parallel testing that includes CSF pleocytosis, increased CSF protein and increased CSF MIF would improve the sensitivity and NPV to avoid missed diagnoses. However, the specificity of CSF MIF for the assessment of NS was only 66.74%, lower than that of CSF pleocytosis (81.40%) and CSF increased protein (86.05%). In contrast, the specificity of the serial testing algorithm (CSF pleocytosis and CSF increased protein) for the assessment of NS was 93.02%, and the specificity increased to 100% when CSF MIF was added to the algorithm. Similarly, in the diagnosis of probable NS, the specificity and PPV of serial testing were both 100%. Thus, serial testing would be a valuable diagnostic indicator of NS, especially in NS patients with negative CSF RPR tests. Serial testing for CSF pleocytosis, increased CSF protein and increased CSF MIF would thus improve the specificity and PPV to avoid misdiagnosis.

The limitations of the present study should also be acknowledged. First, this study was designed as a cross-sectional survey, and no follow-up data for the CSF MIF concentration

were obtained to evaluate the effect of therapy for NS patients. Second, the study was based on 556 syphilis patients, only 16.37% (91/556) of whom received a lumbar puncture; therefore, the incidence of NS among those without CSF assessment was not determined. Third, the sample size was relatively small, so it will be necessary to increase the sample size in future studies.

Our results showed that the sensitivity of CSF MIF for NS screening was higher than that of CSF pleocytosis and CSF increased protein, especially for probable NS patients with negative CSF RPR tests. Parallel testing (positive CSF pleocytosis, positive CSF increased protein and/or positive CSF MIF) would improve the sensitivity and NPV to avoid missed diagnoses, whereas the serial testing algorithm (positive CSF pleocytosis, CSF increased protein and CSF MIF) would improve the specificity and PPV to avoid misdiagnosis. We suggest that the CSF MIF concentration should be used as a novel CSF marker to establish or exclude a diagnosis of NS.

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Table 1

Characteristics of the study subjects.

	NS(n=43) No. (%)	N-NS(n=43) No. (%)	Healthy control(n=63) No. (%)	<i>P</i>
Sex				
Male	29 (67.44%)	24 (55.81%)	38 (60.32%)	# $\chi^2=1.25, P^a=NS$
Female	24 (32.56%)	19 (44.19%)	25 (39.68%)	
Mean age (y)	53.09 (range, 19–85)	50.42(range, 16–80)	50.71(range, 17–85)	* F=0.31, $P^a=0.74$
≥ 50 y	30(69.80%)	20(46.50%)	35(55.60%)	$\chi^2=20.91, P^a=NS$
<50 y	13(30.20%)	23(53.50%)	28(44.40%)	
Sera findings				
Positive RPR	42(97.67%)	26(60.47%)	Neg	# $\chi^2=17.98, P^b=0.00$
Positive TPPA	43(100%)	43(100%)	Neg	
CSF findings				
Positive RPR	17(39.53%)	0(0%)	ND	# $\chi^2=21.18, P^b=0.00$
Positive TPPA	29(67.44%)	0(0%)	ND	# $\chi^2=48.47, P^b=0.00$
Pleocytosis	29(67.40%)	8(18.60%)	ND	# $\chi^2=20.91, P^b=0.00$
Elevated protein	21(48.84%)	6(13.95%)	ND	# $\chi^2=12.14, P^b=0.00$

Abbreviations: NS, Neurosyphilis; N-NS, Syphilis/non-neurosyphilis; RPR, rapid plasma reagin; TPPA, T. pallidum particle agglutination; CSF, cerebrospinal fluid; Neg, negative; ND, not done; n.a, not analyse.

P^a , Among NS, N-NS and healthy control groups; P^b , NS vs. N-NS.

Table 2

The serum and CSF MIF concentration in different groups

	NS (n=43)	N-NS(n=43)	Healthy control(n=63)	NS (n=43)	
				Early NS (n=29)	Late NS (n=14)
Serum-MIF(median [IQR]) (ng/ml)	52.58[28.31-95.94] ^{a,b,c,d}	34.30[19.77-59.75]	11.20[7.89-23.69]	66.41[30.48-96.19] ^e	38.12[24.27-88.90]
CSF-MIF(median [IQR]) (ng/ml)	8.77[4.76-19.13] ^f	4.08[2.21-9.68]	ND	8.09[433-10.80] ^g	21.82[16.46-32.24]

Abbreviations: NS, Neurosyphilis; N-NS, Syphilis/non-neurosyphilis; MIF, macrophage migration inhibitory factor ; ND, not done.

a, Among NS, N-NS and healthy control groups, df=2, P=0.00; b, NS vs. N-NS, z=-2.73, P=0.01; c, NS vs. healthy controls, z=-6.63, P=0.00; d, N-NS vs. healthy controls, z=-4.65, P=0.00; e, early NS vs. late NS, z=-1.17, P=0.24; f, NS vs. N-NS, z=-3.10, P=0.00; g, early NS vs. late NS, z=-5.26, P=0.00.

Table 3

Sensitivity and specificity for clinical diagnosis of NS

	Clinical diagnosis NS(%)					Probable NS(%)					Confirmed NS (%)				
	Sen	Spe	PPV	NPV	ACC	Sen	Spe	PPV	NPV	ACC	Sen	Spe	PPV	NPV	ACC
Positive CSF-RPR	39.53	100	100	62.32	69.77	0	100	n.a	62.32	62.32	100	100	100	100	100
CSF pleocytosis ,> 10 cells/ μ L	67.44	81.40	78.38	71.43	74.42	65.38	75.26	68.00	73.53	71.19	64.71	76.81	40.74	89.83	74.42
CSF elevated protein , >500mg/L	48.84	86.05	77.47	62.71	67.44	53.85	86.05	70.00	75.51	73.91	55.56	68.12	40.54	79.66	64.58
CSF MIF >6.63 ng/ml	74.42 ^{a b c}	67.44	69.57	72.50	70.93	92.31 ^{d e f}	64.10	63.16	92.59	75.38	76.47 ^{g h i}	52.17	28.26	90.00	56.98
Serum MIF>60.49 ng/ml	48.84	76.74	67.74	60.00	62.79	46.15	76.74	54.55	70.21	65.22	52.94	68.12	29.03	85.45	65.12

Abbreviations: NS, Neurosyphilis; Sen, sensitivity; ACC, accuracy; RPR, rapid plasma reagin; n.a, not analyse; MIF, macrophage migration inhibitory factor.

a, CSF MIF vs. CSF RPR, $\chi^2=23.58$, $P=0.00$; b, CSF MIF vs. CSF pleocytosis, $\chi^2=1.18$, $P=0.35$; c, CSF MIF vs. CSF elevated protein, $\chi^2=13.20$, $P=0.00$; sensitivity for probable NS, d, CSF MIF vs. CSF RPR, $\chi^2=170.37$, $P=0.00$; e, CSF MIF vs. CSF pleocytosis, $\chi^2=27.60$, $P=0.00$; f, CSF MIF vs. CSF elevated protein, $\chi^2=36.63$, $P=0.00$; sensitivity for confirmed NS, g CSF MIF vs. CSF RPR, $\chi^2=27.27$, $P=0.00$, h, CSF MIF vs. CSF pleocytosis, $\chi^2=2.91$, $P=0.12$; i, CSF MIF vs. CSF elevated protein, $\chi^2=8.91$, $P=0.00$.

Table 4

Parallel and serial testing algorithms of CSF parameters (RPR, pleocytosis, elevated protein and elevated MIF) for NS screening

Algorithm	Clinical diagnosed NS(%)					Probable NS(%)					Confirmed NS (%)				
	Sen	Spe	PPV	NPV	ACC	Sen	Spe	PPV	NPV	ACC	Sen	Spe	PPV	NPV	ACC
Parallel testing															
CSF pleocytosis or elevated protein	83.72	74.42	76.60	82.05	79.07	80.77	74.42	65.63	86.49	76.81	94.12	55.07	34.04	97.44	62.79
CSF pleocytosis or elevated protein or elevated MIF	97.67	46.51	64.62	95.24	72.09	100	46.51	53.06	100	66.67	100	30.43	26.15	100	44.19
Serial testing															
CSF pleocytosis and elevated protein	32.56	93.02	82.35	57.97	62.79	15.38	93.02	57.14	64.52	63.77	58.82	89.86	58.82	89.86	83.72
CSF pleocytosis and elevated protein and elevated MIF	30.23	100	100	58.90	65.12	37.14	100	100	66.5	71.79	52.94	94.20	69.23	89.04	86.05

Abbreviations: NS, Neurosyphilis; ACC, accuracy;; MIF, macrophage migration inhibitory factor.

Fig.1. The flow of participants from enrolment

Fig. 2. Receiver operating characteristic curves showing sensitivity as a function of specificity in NS and N-NS patients

A, CSF MIF; B, serum MIF