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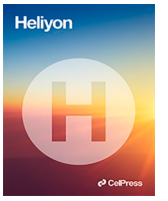

Research article

Neutrophil CD64 index as a potential blood biomarker for the diagnosis of neurosyphilis in secondary and tertiary syphilis: A retrospective study

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ABSTRACT

Objective: To examine the correlation of neutrophil CD64 (nCD64) index with neurosyphilis (NS) across different stages of syphilis.
Methods: A total of 1243 syphilis patients at different stages (344 of primary, 385 of secondary, and 514 of tertiary) included in this study were divided into NS and non-NS (NNS). Correlations of nCD64 index with currently used syphilis biomarkers were explored using Spearman correlation test. Relationships between nCD64 index and NS at different stages were investigated by stratified analysis and restricted cubic spline model. The diagnostic performance of nCD64 index for NS was assessed by receiver operating characteristic (ROC) curve.
Results: Significant statistical correlations of nCD64 index with cerebrospinal fluid (CSF) NS indicators were found in secondary and tertiary syphilis. Increased nCD64 index was associated with increased risk of NS in secondary and tertiary syphilis. ROC analysis values further confirmed the diagnostic potential of nCD64 index for NS. Marked decrease of nCD64 index was observed in NS patients after effective antisyphilitic treatments.
Conclusions: The nCD64 index may help to the diagnosis of NS in secondary and tertiary syphilis.

1. Introduction

Syphilis is a common sexually transmitted disease (STD), which is caused by the spirochete *Treponema pallidum* (T. *pallidum*, TP) subspecies *pallidum* [1,2]. It usually causes multi-organ lesions and remains a worldwide public health concern [1,2]. There were about

Abbreviations: nCD64, neutrophil CD64; NS, neurosyphilis; NNS, non-neurosyphilis; ROC, receiver operating characteristic curve; CSF, cerebrospinal fluid; STD, sexually transmitted disease; TP, *Treponema pallidum*; WHO, World Health Organization; CNS, central nervous system; WBC, white blood cells; PRO, protein; TPPA, treponema pallidum gelatin particle agglutination test; VDRL, venereal disease research laboratory test; RPR, rapid plasma reagin test; TRUST, toluidine red unheated serum test; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; FcγRI, immunoglobulin G Fc receptor I; HSV, herpes simplex virus; MFI, mean fluorescent intensity; IQR, interquartile ranges; RCS, restricted cubic splines; AUC, area under ROC curve; CI, confidence interval; OR, odds ratio.; Neutrophil CD64 index as a potential blood biomarker for the diagnosis of neurosyphilis in secondary and tertiary syphilis, A retrospective study.

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7.1 million adults aged 15 to 49 acquired syphilis in 2020 as reported by the World Health Organization (WHO) (https://www.who.int/news-room/fact-sheets/detail/syphilis). In China, new cases of syphilis reached 497934 in 2022, increased by 17914 compared to the number of 2021 (https://data.stats.gov.cn). Clinical progression of syphilis usually comprises a primary chancre at the site of inoculation, followed by the non-itchy rash on the palms and soles as the secondary syphilis, a tertiary period with no symptoms, and the tertiary syphilis with the involvement of the eyes, central nervous system (CNS), and cardiovascular systems *etc.* [3]. Neurosyphilis (NS) is elicited by the invasion of TP into the CNS and is the most serious and common complication of syphilis [4]. NS can occur at any stage of syphilis and symptoms include meningitis, meningovascular disease, or parenchymal syphilis (including tables dorsalis and general paresis) *etc.* [2]. The incidence of NS is about 7.1% in primary syphilis, 23.8% in secondary syphilis, and 26.6% in tertiary syphilis [5]. Once NS happened, the injury to neurons is irreversible and life quality of the syphilis patients will be greatly affected. Therefore, early detection and timely intervention is crucial for NS patients.

Laboratory diagnosis of NS is mainly based on the examinations of anti-TP antibodies, white blood cells (WBC) counts and protein (PRO) levels in cerebrospinal fluid (CSF) [4]. Detection of the anti-TP antibodies includes the specific treponema pallidum gelatin particle agglutination (TPPA) test and the nonspecific nontreponemal test. For the latter one, there are venereal disease research laboratory (VDRL) test, rapid plasma reagin (RPR) test and toluidine red unheated serum test (TRUST) *etc*. The recommended non-treponemal test in CSF is VDRL, while RPR and TRUST were reported to have comparable diagnostic performance with VDRL [6]. However, no one test can definitively confirm or exclude NS due to the defects in specificity and sensitivity [7]. Besides, some patients with no-obvious symptoms usually refuse to take the lumbar puncture considering the invasive operation, which may lead to the missed detection of early and asymptomatic NS [8,9]. What's more, as the great imitator, neurosyphilis can mimic many psychiatric and neurological diseases and leads to the diagnostic delay in patients without previous known history of syphilis [10,11]. Thus, it is in great demand to look for other indicators which are easy to determine and have better performance in NS diagnosis.

As the first line of defense in innate immune system, neutrophils play pivotal roles in protecting against infectious pathogens and inflammatory agents. In response to infections and pro-inflammatory cytokines like interferon (IFN)-γ, interleukin (IL)-6, tumor necrosis factor (TNF)-α, and granulocyte colony-stimulating factor (G-CSF), the high-affinity immunoglobulin G Fc receptor I (FcγRI, CD64) is rapidly induced on neutrophils [12]. CD64 functions as an activating FcγR via a cytoplasmic immunoreceptor tyrosine-based activation motif, which results in immune effector functions such as phagocytosis, antigen presentation, antibody-dependent cellular cytotoxicity and mediator secretion [13]. Therefore, neutrophil CD64 (nCD64) index has been a reliable marker for infectious diseases such as sepsis [14], mycobacterium tuberculosis infection [15], community-acquired pneumonia [16], and COVID pneumonia [17] *etc*. Despite these developments, the potential application of nCD64 index in the diagnosis of syphilis remains unknown.

In this retrospective study, we reviewed clinical data from 1243 syphilis patients and found nCD64 index was significantly correlated with currently used NS markers in secondary and tertiary syphilis. Further exploration identified the diagnostic potential of nCD64 index for NS in secondary and tertiary syphilis. Decreased nCD64 index was observed in NS patients after effective treatments. These findings proposed the potential value of peripheral nCD64 index in diagnosing NS among secondary and tertiary syphilis.

2. Materials and methods

2.1. Study population

A total of 1243 patients diagnosed with syphilis in Shanghai Skin Disease Hospital from 2020 January to 2023 September were included in this study. The research was approved by the Ethics Committee of the hospital (Approval No: SSDH-IEC-SG-080-4.0; approval date: 2023-09-10). Patients with sepsis, mycobacterium tuberculosis infection, COVID-19, malignant tumors, HIV and systemic inflammatory diseases were excluded.

2.2. Diagnostic criteria for syphilis

According to the WHO guidelines for the treatment of TP [1] and previous studies [18], diagnostic criteria for syphilis were as followed. *Primary syphilis*: (1) chancres or ulcers; and/or (2) detection of spirochetes in a dark-field microscopy examination; and (3) positive serum TRUST and TPPA; and (4) absence of other causes of genital ulcers, including herpes simplex virus (HSV) infections; and (5) time after TP infection $\leq$3 months. *Secondary syphilis:* (1) positive serum TRUST and TPPA; (2) skin or mucocutaneous lesions; (3) 3 months $<$ time after TP infection $\leq$2 years. *Tertiary syphilis:* (1) positive serum TRUST and TPPA; (2) with or without visible skin or mucocutaneous lesions; (3) time after TP infection $>$2 years. The antisyphilitic treatment regimen typically involves benzathine penicillin (2.4 MU/qw im for 2 or 3 weeks) or procaine penicillin (0.8 MU/day im for 15 days) in most cases.

2.3. Diagnostic criteria for NS

The diagnosis of NS was based on the medical history, physical and laboratory examinations of the patients. *NNS*: (1) serum TPPA and TRUST positive; (2) CSF TPPA and TRUST negative; (3) no clinical manifestations of NS. *NS:* (1) serum TPPA and TRUST positive; (2) CSF TPPA positive (positive TPPA due to past infection was excluded); (3) Meet one of the three: CSF WBC $\geq$5 cells/μL, CSF protein $\geq$450 mg/L or CSF TRUST titer$>$0; (4) without or with clinical symptoms of neurologic disorder without other causes. The symptoms include paralytic dementia, syphilitic meningitis, and psychiatric symptoms such as personality changes, cognitive decline *etc.*

2.4. Variables and measurements

Neutrophil CD64 expression was determined by flow cytometer (BD Pharmingen, San Diego, USA). The calculation formula was as followed: nCD64 index $= \frac{CD64MFI(PMN)/CD64MFI(LYM)}{CD64MFI(MO)/CD64MFI(PMN)}$, MFI refers to mean fluorescent intensity. Serum and CSF TRUST titers were determined by the commercial kits (Rongsheng, Shanghai, China). CSF TPPA test was carried out using the TP antibody detection kit (Fujirebio Inc, Tokyo, Japan). WBC in CSF were counted with the Neubauer's counting chamber under a microscope (Shanghai, China). CSF protein was detected by an automatic biochemical analyzer (Roche Cobas e702, Mannheim, Germany).

2.5. Statistical analysis

MedCalc v20.218, R version 4.1.2 and GraphPad Prims 8.0 were used for statistical analysis. Categorical variables were expressed

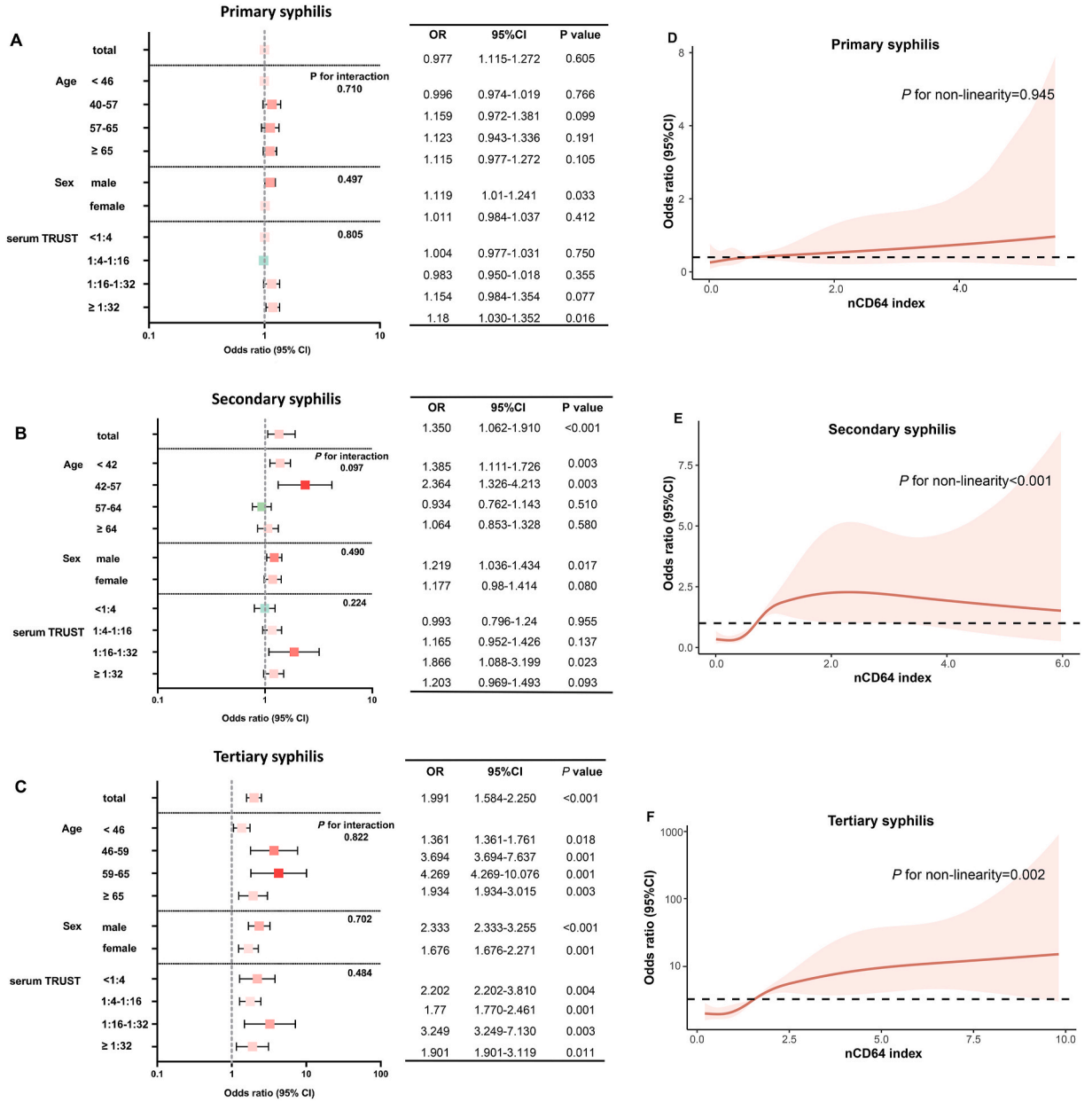

Fig. 1. Association of nCD64 index with NS. Forest map of logistic regression for nCD64 index and NS in primary (A), secondary (B) and tertiary syphilis (C) was plotted according to subgroups divided by IQR. The ***total*** on the top of each figure represented the individual effect of nCD64 index on NS. Trend fitting between nCD64 index and NS was performed using restricted cubic splines (D–F).

as case numbers and percentages (%), and continuous variables were presented as medians and interquartile ranges (IQR). The χ^2 test was used for comparison between categorical variables. Mann-Whitney test was used for two groups of continuous variables. Correlations of nCD64 index with serum and CSF biomarkers were explored by Spearman correlation test. Association between nCD64 index and NS was assessed using stratified analysis. First, subgroup analysis according to age, sex and serum TRUST was performed using logistic regression. Second, Jonckheere-Terpstra trend test and restricted cubic splines (RCS) for patients within 95% confidence interval (CI) were used to visualize the relationship between nCD64 index and NS occurrence. Clinical diagnostic performance of each indicator was assessed by the receiver operating characteristic (ROC) curve. Area under the curve (AUC) was compared by DeLong's test. Comparison of markers before and after treatment was performed via paired *t*-test. In this research, age and sex were considered as potential confounders. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics of syphilis patients enrolled

A total of 1243 patients including 344 primary syphilis, 385 secondary syphilis and 514 tertiary syphilis were included in this study (Supplementary Fig. 1). Demographic and clinical characteristics of these patients were listed in Supplementary Tables 1–3. Among them, 847 patients were diagnosed as NS, with 133 patients at primary stage, 290 at secondary stage, and 424 at tertiary stage. Of the NS patients, there were 190 cases with paralytic dementia, 38 cases with tuberculosis spinal cord, 21 cases with late ocular syphilis, 107 cases with meningeal NS, and 492 cases with asymptomatic NS (ANS). In all three stages, NS patients were generally older than NNS patients (primary $P < 0.001$, secondary $P < 0.001$, tertiary $P = 0.002$). Besides, more male than female NS patients were observed (primary $P < 0.001$, secondary $P < 0.001$, tertiary $P = 0.017$), which was consistent with the epidemiological characteristics of syphilis [19]. No significant difference of serum TRUST titers was found between NS and NNS patients in primary and secondary syphilis, while NS patients exhibited substantially higher serum TRUST titers than the NNS patients in tertiary syphilis ($P < 0.001$) (Supplementary Tables 1–3). CSF TRUST titers ($P < 0.001$), WBC ($P < 0.001$) and PRO ($P < 0.001$) of NS patients were significantly higher than those of NNS at all three stages (Supplementary Tables 1–3).

3.2. Correlation analysis of nCD64 index with currently used NS biomarkers

Currently, the diagnosis of NS usually depends on examinations of serum TRUST, CSF TRUST, CSF WBC, and CSF PRO. Thus, we first investigated the correlations of nCD64 index with these markers (Supplementary Table 4). Results indicated that nCD64 index was significantly correlated with serum TRUST titer, which reflects the activity and infectivity of syphilis, in both secondary syphilis ($r = 0.170$, $P < 0.001$) and tertiary syphilis ($r = 0.162$, $P < 0.001$). Similar positive correlations of nCD64 index with CSF biomarkers, which indicate neuro-invasion, were observed in secondary syphilis (CSF TRUST $r = 0.148$, $P < 0.001$; CSF PRO $r = 0.188$, $P < 0.001$; CSF WBC $r = 0.200$, $P < 0.001$). Much stronger correlations of nCD64 index with these biomarkers were found in tertiary syphilis (CSF TRUST $r = 0.326$, $P < 0.001$; CSF PRO $r = 0.253$, $P < 0.001$; CSF WBC $r = 0.258$, $P < 0.001$), while no significant correlations were found in primary syphilis.

3.3. Correlation of nCD64 index with NS

Significantly increased nCD64 indices were found in NS patients of secondary and tertiary syphilis but not primary syphilis (Supplementary Tables 1–3). To explore the relationship between nCD64 index and NS, stratified analysis was conducted. In general, increased nCD64 index was correlated with higher possibility of NS in secondary syphilis (OR = 1.350, 95%CI = 1.062–1.910, $P < 0.001$, Fig. 1B). In tertiary syphilis, the risk of having NS increased by 99.1% with each 1% increase of nCD64 index (OR = 1.991, 95% CI = 1.584–2.250, $P < 0.001$, Fig. 1C), and subgroup analysis according to age, sex and serum TRUST titer showed consistently strong

Table 1
Trend test for the association between nCD64 index on neurosyphilis.

	nCD64 index	OR	95% CI	*P*	*P* for trend
Primary syphilis	<0.55	2.224	1.023–83.169	0.057	0.011
	0.55–1.31	1.638	0.62–11.225	0.189	
	1.31–1.60	1.374	0.613–3.081	0.44	
	≥1.60	1.073	0.98–1.174	0.127	
Secondary syphilis	<0.56	0.923	0.221–4.783	0.033	<0.001
	0.56–1.37	2.68	0.446–16.078	0.028	
	1.37–2.93	2.338	0.649–8.416	0.019	
	≥2.93	1.159	0.899–1.493	0.025	
Tertiary syphilis	<0.79	1.076	0.190–6.064	0.933	<0.001
	0.79–2	5.243	1.561–17.605	0.007	
	2–3.7	33.553	0.973–1156.442	0.021	
	≥3.7	34.39	10.840–2102.300	0.039	

Table shows the odds ratio and 95%CI of each subgroup divided by IQR of nCD64 index of each stage. *P* values for Jonckheere-Terpstra trend tests examined whether increased levels of nCD64 index associate with NS.

correlations (Fig. 1C). However, changes of nCD64 index had no significant correlation with NS in primary syphilis (OR = 0.977, 95% CI = 1.115–1.272, $P = 0.605$) (Fig. 1A).

The relationship of nCD64 index with NS was further visualized using trend test (Table 1) and RCS analysis adjusted by age and sex (Fig. 1D–F). In primary syphilis, the relationship between nCD64 index and NS showed a positive correlation trend (P for trend = 0.011, Table 1), and the RCS showed a smooth curve though with relatively low OR (P for non-linearity = 0.945, Fig. 1D). In secondary syphilis, a positive relationship was found (P for trend<0.001, Table 1) and the estimated curve showed increased OR when nCD64 index increased (P for non-linearity<0.001, Fig. 1E). In tertiary syphilis, nCD64 was found to be strongly correlated with NS when it was larger than 0.79 (P for trend<0.001, Table 1). Consistently, the RCS curve showed a growing OR for NS with the increase of nC64 index (P for non-linearity = 0.002, Fig. 1F).

3.4. Diagnostic performance of nCD64 index in NS

We then proceed to figure out whether nCD64 index had potential value in NS diagnosis. ROC curves of different markers for NS diagnosis were plotted, the AUC value of nCD64 index in primary syphilis was 0.586 (95%CI = 0.530–0.640), which was not statistically different with those of serum TRUST and CSF biomarkers (Fig. 2A, Table 2). In secondary syphilis, the AUC value of nCD64 index in diagnosing NS was 0.674 (95%CI = 0.616–0.728), which was better than that of serum TRUST (AUC = 0.568, 95%CI = 0.531–0.638, DeLong's test $P = 0.043$) and was on par with those of CSF TRUST (AUC = 0.689, 95%CI = 0.632–0.743, DeLong's test $P = 0.733$), CSF PRO (AUC = 0.710, 95%CI = 0.653–0.762, DeLong's test $P = 0.469$) and CSF WBC (AUC = 0.583, 95%CI = 0.523–0.614, DeLong's test $P = 0.065$) (Fig. 2B–Table 2). Remarkably, the AUC value of nCD64 index for NS diagnosis in tertiary syphilis increased to 0.792 (95%CI = 0.751–0.830), which significantly outperformed those of serum TRUST (AUC = 0.635, 95%CI = 0.588–0.680, DeLong's test $P < 0.001$), CSF TRUST (AUC = 0.703, 95%CI = 0.657–0.745, DeLong's test $P = 0.025$), CSF PRO (AUC = 0.510, 95%CI = 0.462–0.558, DeLong's test $P < 0.001$) and CSF WBC (AUC = 0.671, 0.624–0.725, DeLong's test $P = 0.004$) (Fig. 2C–Table 2).

To further identify the correlation of nCD64 index with TP activity in NS, we compared the data of NS patients who had nCD64 index determination before and after antisyphilitic treatments. A total of 54 NS patients were screened out. According to their follow-up assessments, serum TRUST titers of these patients decreased to lower than 4 within two months. Meanwhile, nCD64 index dropped significantly after antisyphilitic treatments ($P < 0.001$) (Fig. 3).

4. Discussion

By retrospectively reviewing the clinical data of 1243 syphilis patients, we comprehensively analyzed the value of nCD64 index in NS diagnosis across syphilis patients at different stages in the present study. Our findings revealed that nCD64 index was positively correlated with currently used indicators of NS and significantly increased nCD64 index was observed in NS patients of secondary and tertiary syphilis. Moreover, increased nCD64 index indicated increased possibility of NS. The diagnostic performance of nCD64 index for NS in secondary and tertiary syphilis was on par or superior to the currently used CSF biomarkers.

The diversity of symptoms or lack of symptoms of NS patients usually makes the diagnosis of NS difficult or even overlook. Of symptomatic cases, neurosyphilis could mimic many other neurological diseases, which may lead to significant diagnostic delay in patients without previous known history of syphilis. The most common clinic neurosyphilis manifestations of dementia, psychosis, and cognitive impairment are also observed in primary psychosis [20] and neurodegenerative diseases such as Alzheimer's disease [21], Parkinson's Disease and Huntington Disease *etc* [10,22]. Hence, the laboratory examination is of great importance for the diagnosis and differential diagnosis of neurosyphilis. At present, the most used indicators were serum and CSF anti-TP antibodies, CSF WBC and CSF protein levels [23,24]. However, there are NS patients with negative CSF TRUST/VDRL [25] and asymptomatic NS patients who do not accept the lumbar puncture for the consideration of its invasiveness and safety. Therefore, lots of researchers turn to explore new biomarkers in blood. Xie et al. reported that serum ubiquitin C-terminal hydrolase-L1 (UCH-L1), glial fibrillary acidic protein (GFAP) and neurofilament light protein (NF-L) had pleasant performances in the diagnosis of NS [26,27]. Shen et al. found that

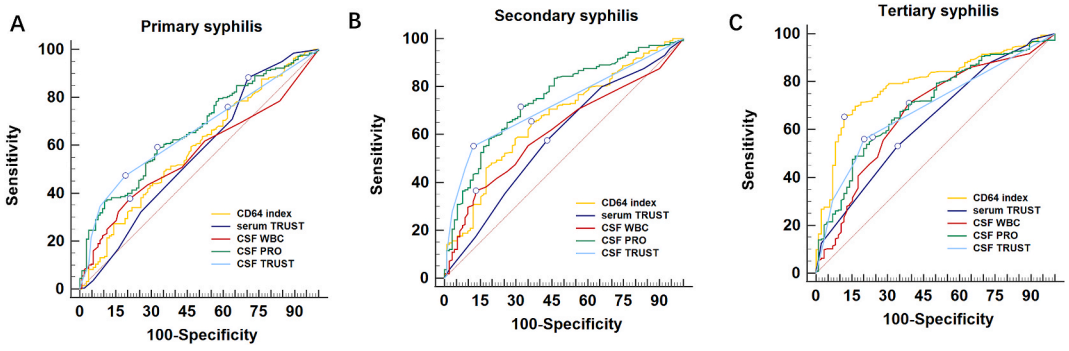

Fig. 2. Diagnostic capabilities of nCD64 index and currently used neurosyphilis diagnostic indicators.

Table 2
Comparison of AUC of nCD64 index and syphilis biomarkers for syphilis.

		AUC	95%CI	DeLong's test *P*	Youden index
Primary syphilis	nCD64 index	0.586	0.530–0.640		1.56
	Serum TRUST	0.566	0.510–0.621	0.682	2
	CSF TRUST	0.663	0.609–0.715	0.109	0
	CSF PRO	0.547	0.491–0.602	0.433	434.5
	CSF WBC	0.562	0.506–0.617	0.618	6
Secondary syphilis	nCD64 index	0.674	0.616–0.728		1.91
	Serum TRUST	0.568	0.531–0.638	0.043	8
	CSF TRUST	0.689	0.632–0.743	0.733	0
	CSF PRO	0.71	0.653–0.762	0.469	417
	CSF WBC	0.583	0.524–0.641	0.065	9
Tertiary syphilis	nCD64 index	0.792	0.751–0.830		8
	Serum TRUST	0.635	0.588–0.680	<0.001	8
	CSF TRUST	0.703	0.657–0.745	0.025	0
	CSF PRO	0.51	0.462–0.558	<0.001	487.9
	CSF WBC	0.671	0.624–0.715	0.004	3

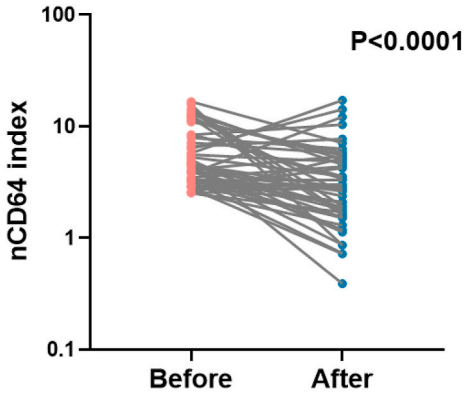

Fig. 3. Comparison of nCD64 index in neurosyphilis patients before and after treatment.

circulating IL-26 in serum displayed diagnostic potential in the progression of NS [28]. Chen et al. analyzed the exosomal miRNA profiles in the serum of syphilis patients, and found NS patients showed a significant upregulation in miR-590-5p, miR-570-3p and miR-570-5p [29]. However, all these markers are still at the research stages, which lack confirmation of large clinic samples, standardized detecting systems and mature commercial kits. Thus, we try to find whether there are other in-use blood indicators which could help the diagnosis of NS.

Immune cells play critical roles in the elimination of various pathogens. During this process, the quantities, activation status and products of immune cells vary a lot, which may be potential biomarkers for the diagnosis and therapeutic monitoring of diseases. In fact, peripheral WBC counts, lymphocyte subsets analysis and cytokines determination are widely used in infectious and inflammatory diseases. As an integral part of WBC counts, neutrophils work as the first line against pathogens. Recently, CD64 expression on neutrophils in infectious diseases has aroused great interests of researchers. CD64 (FCγR1) is the Fc receptor that binds to monomeric IgG type antibodies with high affinity, which makes it important in phagocytosis. Normally, CD64 is mainly expressed on the surface of macrophages, monocytes and dendritic cells. However, nCD64 increases rapidly in response to microbial wall components, complement split products and some pro-inflammatory cytokines [12,30]. Thus, nCD64 had been used in the diagnosis and prognosis of several infectious diseases [31–34]. Although neutrophils are reported to play important roles in STD such as mycoplasma [35], chlamydia [36] and gonococcus [37] infection, and inhibition of neutrophils can lead to immune evasion of Chlamydia [38] and Treponema denticola [39], the diagnostic potential of nCD64 index in syphilis has not been well elucidated. In our study, significantly increased nCD64 index was observed in NS patients of secondary and tertiary syphilis but not primary syphilis. According to the binary logistic regression analysis, increased nCD64 index also suggested increased possibility of NS in secondary and tertiary syphilis. These results indicated nCD64 index had potential value in NS diagnosis of syphilis patients at mid to late stages.

When exploring the diagnostic performance of nCD64 index in NS, we first evaluated its correlation with the currently used indicators. It turned out that nCD64 index was positively correlated with serum and CSF TRUST titers, CSF WBC counts and CSF protein levels in secondary and tertiary syphilis, although with low correlation coefficients. AUC value analysis indicated that nCD64 index had comparable diagnostic performance with the currently used biomarkers for NS in secondary syphilis, and markedly superior performance in tertiary syphilis. Hence, nCD64 index could be used as a potential peripheral substitute for CSF indicators in NS diagnosis in secondary and tertiary syphilis, especially for those without typically clinical manifestations and do not accept the lumber puncture. However, for NS diagnosis in primary syphilis patients, nCD64 index alone was not enough. At the early stages of infection,

the strong activation of neutrophils and the resulting acute inflammatory responses work together to clear the sudden invaded and replicated TP, which exhibited no significant difference between the NS and NNS of primary syphilis patients. In syphilis cases at mid-to-late stages, the anti-TP immune responses turn to mainly acquired immunity. Once the activities of TP increase and TP invade into the CNS, neutrophils may be reactivated and try to protect the host, which leads to the changes of nCD64 index.

In summary, our study demonstrated for the first time that nCD64 index may benefit the diagnosis of NS patients in secondary and tertiary syphilis. Given the risks of lumbar puncture and limitations of existing laboratory biomarkers, nCD64 index offers a less invasive alternation and holds significant promise for improving the diagnosis of NS patients. Moreover, the findings of this study provide valuable insights into the host's immune responses towards syphilis infection, shedding light on the different immune response dynamics at various stages of the disease. Further study on the underlying mechanisms is needed in future research.

Ethical approval statement

The study was approved by the Ethics Committee of Shanghai Skin Disease Hospital (Approval No: SSDH-IEC-SG-080-4.0; approval date:2023-09-10). We confirmed that all the data were anonymized and maintained with confidentiality. Given the retrospective nature of our study, we did not alter the standard treatment protocols for enrolled patients. Furthermore, due to the nationwide distribution of the participants, obtaining written informed consent from all patients proved to be impractical. Consequently, we opted for verbal consent via telephone.

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Data availability

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

CRediT authorship contribution statement

Yijie Tang: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lingyun Shen:** Data curation. **Dandan Yang:** Data curation. **Jiaqin Zhang:** Data curation. **Qinghui Xie:** Data curation. **Fenyong Sun:** Writing – review & editing. **Qingqiong Luo:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29027.

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