Iran J Public Health, Vol. 44, No.2, Feb 2015, pp.155-169

Review Article

Potential Predictors for Serofast State after Treatment among HIV-Negative Persons with Syphilis in China: A Systematic Review and Meta-Analysis

***Jiabi QIN** [1, 2], ***Tubao YANG** [3], **Hua WANG** [1], **Tiejian FENG** [4], **Xiaoying LIU** [5]

1. Division of Medical Genetics, Maternal and Child Health Hospital of Hunan Province, Hunan, China
2. Skate Key Laboratory of Medical Genetics, Central South University, Hunan, China
3. Dept. of Epidemiology and Health Statistics, School of Public Health, Central South University, Hunan, China
4. Dept. of Dermatology and Venereal Disease, Shenzhen Center for Chronic Disease Control and Prevention, Shenzhen, China
5. Caimao Hospital of Hunan Province, Hunan, China

***Corresponding Author:** Email: qinjiabi123@163.com, yangtb@xysm.net

(Received 04 Aug 2014; accepted 11 Dec 2014)

Abstract

Background: Several studies have been conducted in China in order to investigate the potential predictors of serofast state after treatment among syphilitic patients. However, there is a remarkable diversity among the results. This meta-analysis was conducted to assess potential predictors of serofast among syphilitic patients in China.

Methods: International and national electronic databases were searched up to September 2013. Reference lists of retrieved articles were also reviewed. Cohort or case-control studies addressing risk factors of serofast among syphilitic patients were included in this study.

Results: We assessed 27 separate studies involving overall 6682 HIV-negative participants with syphilis of which 1962 remained in the serofast state. The serofast was positively associated with older age(P_{trend}=0.001), female(summary risk ratio[sRR]=1.50, 95%CI:1.34-1.68), latent syphilis($sRR_{latent\ vs\ primary}$=3.17, 95%CI: 2.66-3.77; $sRR_{latent\ vs\ secondary}$=2.00, 95%CI: 1.48-2.69) as well as non-penicillin treatment(sRR =2.99, 95%CI:2.45-3.67), but negatively associated with higher baseline titers($sRR_{>1:32\ vs\ \leq 1:32}$=0.63, 95%CI: 0.54-0.75). Compared with healthy group and serological cure group, respectively, the levels of CD4 (+), IL-2, and IL-6 among serofast patients were decreased (standardized mean difference[SMD]<0, *P*<0.05), but the levels of CD8(+) and IL-10 were increased(SMD>0, *P*<0.05). Some studies also hinted the serofast was associated with subtypes i of treponema pallidum(TP) repeat gene ($RR_{i\ vs\ d}$=4.67,95%CI: 1.31-16.69) and TP occult infection.

Conclusion: The age, gender, stage of infection, baseline titers, treatment drug, cellular immune suppression and disorders, TP occult infection and subtypes i of TP repeat gene should be considered as important predictors of serofast. However, until now the definition and mechanism of serofast has still been not clear.

Keywords: Syphilis, Serofast, T-lymphocyte subset, Interleukin, China, Meta-analysis

Introduction

Syphilis has been a major cause of mortality and morbidity for around 500 years (1). The World Health Organization (WHO) estimates that 12 million new cases of syphilis occur each year (2, 3). In Chinese history, syphilis had been eradicated over a period in 1960s, but by the late 1990s, China's reported syphilis cases were rising by about 30% per year (4). The reported incidence of syphilis more than tripled between 2005 and 2011, to 32.0 cases per 100 000 people. This increasing

trend was continuously observed in 2012 (5, 6). Facing the continually increasing epidemic of syphilis in China, the government has recognized that enhanced efforts are needed to respond to the epidemic. Specifically, in 2010, the China's Ministry of Health (MOH) officially launched the first national program specially and directly aimed at controlling syphilis: the National Program for Prevention and Control of Syphilis in China (2010–2020)(7). However, the spread of syphilis transmission seem not to be prevented actually. Worriedly, a new problem of the persistent positive serological reaction after treatment among syphilitic patients is coming with the spread of syphilis, which is bound to bring about new difficulties for prevention of syphilis transmission.

Syphilis management requires serological monitoring after therapy (8-12). "Not all patients achieve serological reversal after the recommended treatment; some patients demonstrate a persistent positive serological reaction that was quite disconcerting for both the physician and patient" (13). It remains unclear whether the persistent positive serological reaction indicates persistent foci of spirochetes or progressive syphilitic lesions or whether it reflects the persistence of regain in the circulating blood following anti-syphilitic therapy. For these reasons, a discussion about the serological response after the recommended therapy is more than justified. In China, in a substantial proportion(10.0% for primary syphilis, 17.5% for secondary syphilis, and 40.5% for latent syphilis) of patients with syphilis, nontreponemal titers neither increase nor decrease 4-fold after treatment and are referred to as being "serofast"(14-18). Until now, the factors that predict the serological response after the recommended therapy among syphilitic patients have not yet been thoroughly studied. A clear understanding of predictors is important to define interventions in every community. With recently accumulating evidence, our goal, therefore, was to summarize factors associated with serofast state of HIV-negative patients with syphilis in China by conducting a meta-analysis of published studies, which serves to guide clinicians regarding the identification of patients who may become serofast after therapy without clear evidence of treatment failure, and develop the optimal management program of serofast patients.

Methods

Literature search

We attempted to report this meta-analysis in accordance with the Meta-Analysis of Observational Studies in Epidemiology guidelines (19). We used and combined the following keywords: "(Serofast OR Seroresistance OR Serum fixed OR Serological cure OR Serological response) AND Syphilis AND China". We searched both international and national electronic databases as follows: PubMed (January 1950 to September 2013), Google Scholar (January 1950 to September 2013), Chinese National Knowledge Infrastructure (CNKI) (January 1994 to September 2013), Chinese Scientific Journals Fulltext Database (CQVIP)(January 1989 to September 2013), China Biology Medicine disc(CBMdisc)(January 1978 to September 2013), and Wanfang Data(January 1998 to September 2013). We also scanned the reference lists of all included studies for additional references. The grey literatures and conference abstracts were not searched. When reported data were not sufficient for estimation of desired comparisons, we contacted study authors.

Selection criteria

We first performed an initial screening of titles or abstracts. A second screening was based on full-text review. Studies were considered eligible for inclusion if they met the following criteria: 1) the study assessed the risk factors of serofast; 2) the study was published in Chinese or English language; 3) study participants were Chinese populations with syphilis but without HIV infection; 4) definition of serofast was clear; 5) all syphilitic patients received recommended therapy according to CDC guidelines(8) before diagnosed as serofast; 6) the exposure of interest was clinical characteristics, the levels of T-lymphocyte subset and NK cells in the peripheral blood, and the levels of interleukin and interferon in the serum; 7) the outcome of interest was serofast; 8) corresponding data were

provided; 9) the study design was a cohort or case-control study. We excluded review papers, non-peer-reviewed local/government reports, conference abstract and presentation in the present study. Multiple papers from the same center and/or authors were analyzed to determine whether the most recent publication was an accumulation, which included cases, reported in earlier publications. If this was evident from our review, then we used only the most recent publication. We also assessed potential studies to ensure that there was no duplication of case series.

Group definitions and measure of exposure

After recommended therapy (non–penicillin-allergic participants were given 3 injections of 2.4 million units of benzathine penicillin at weekly intervals; penicillin-allergic participants were given doxycycline or azithromycin or erythromycin), syphilis patients were considered to be in a serofast state if their nontreponemal antibodies test remained positive and the titres neither increased nor decreased by at least four-fold (two dilutions)(9, 10). Syphilis patients whose clinical manifestations disappeared and whose nontreponemal antibodies titers became negative or decreased by four-fold(two dilutions) were regarded as achieving a serological cure(9, 10). Health group consisted of populations who went to the hospital to donate blood or seek a physical examination. They were not infected with syphilis and HIV. The Flow cytometry was used to detect the levels of T-lymphocyte subset and NK cells in the peripheral blood among health group, serofast group and serological cure group. The Enzyme linked immunosorbent assay (ELISA) was also used to detect the levels of Interleukin and interferon in the serum among healthy group, serofast group and serological cure group.

Data extraction and quality assessment

Two independent reviewers (QJB and YTB) extracted data and assessed study quality. Any disagreements were resolved through discussion among the authors until consensus was reached. Data extraction was then performed using a standardized data-collection form. For cohort studies, the following data were extracted: first author and year of publication; study location; study design/period; duration of follow-up; study population; exposure and outcome; methods of data collection; and comment. For case-control studies, the following data were extracted: first author and year of publication; study location; study design/period; case group/sample size; control group/sample size; exposure; measure of exposure; and comment.

We adapted the principles of the Newcastle-Ottawa-Scale (NOS) to assess the risk of bias in the included studies (20). In statistics, the scale is a tool used for assessing the quality of non-randomized studies included in a systematic review and/or meta-analysis. Using the tool, each study is judged on eight items, categorized into three groups: the selection of the study groups; the comparability of the groups; and the ascertainment of outcome or exposure. Stars awarded for each quality item serve as a quick visual assessment. Stars are awarded such that the highest quality studies are awarded up to nine stars. When the study gains at least six stars, it is considered of low-risk of bias (21). We only included studies with low-risk of bias.

Statistical analysis

The summary risk ratio (sRR) and standardized mean difference (SMD), respectively, were used for qualitative data and quantitative data to measure of the association between serofast and its potential risk factors, and odd ratios and incidence rate ratios were directly considered as RRs. Homogeneity of effect size across studies was tested by using the Q statistics at the $P<0.10$ level of significance. The I^2 statistic, which is a quantitative measure of inconsistency across studies, was also calculated (significance level at $I^2>50\%$) (22). sRR or SMD and their corresponding 95%CI were calculated using either fixed-effects models or, in the presence of heterogeneity, random-effects models (23). Sensitivity analysis was conducted to explore possible explanations for heterogeneity and examine the influence of various exclusion criteria on the overall risk estimate. We also investigated the influence of a single study on the overall risk esti-

mate by omitting one study in each turn. Potential publication bias was assessed by visual inspection of the Begg's funnel plots, Begg's rank correlation test, Egger's linear regression test, and Macaskill's test (24). Begg's rank correlation test, Egger's linear regression test, and Macaskill's test were performed by using SAS version 8.2(SAS Institute, Cary, NC, USA). Other analyses were performed by Review Manager-version 5.0. A *P*value <0.05 was considered statistically significant, except where otherwise specified.

Results

Study selection and study quality assessment

Overall, 1245 studies were identified, of which 402 potentially relevant articles were selected for further screening, and eventually 27 studies (10,11,14,17,18,25-46) involving 6682 HIV-negative participants with Syphilis in China were considered eligible for inclusion (Fig.1). The extracted data involving 11 cohort studies and 16 case-control studies and published between 2005 and 2013 were shown in Table 1. The shortest length from first treatment to diagnosis of serofast after treatment was more than 6 months, and the length in 92.6 %(25/27) of eligible studies was ≥ one year. In all studies, blood sample were tested for HIV and syphilis. Some studies comprising participants with positive HIV test have been excluded. In included studies, participants with serum positive for both *Treponema pallidum* particle assay(TPPA) and rapid plasma regain(RPR) or toluidine red unheated serum test(TRUST) were determined to be currently infected with syphilis. RPR or TRUST titers were used to evaluate serological response at 6 and 12 months after treatment for primary and secondary syphilis and, additionally, at 24 months for latent syphilis.

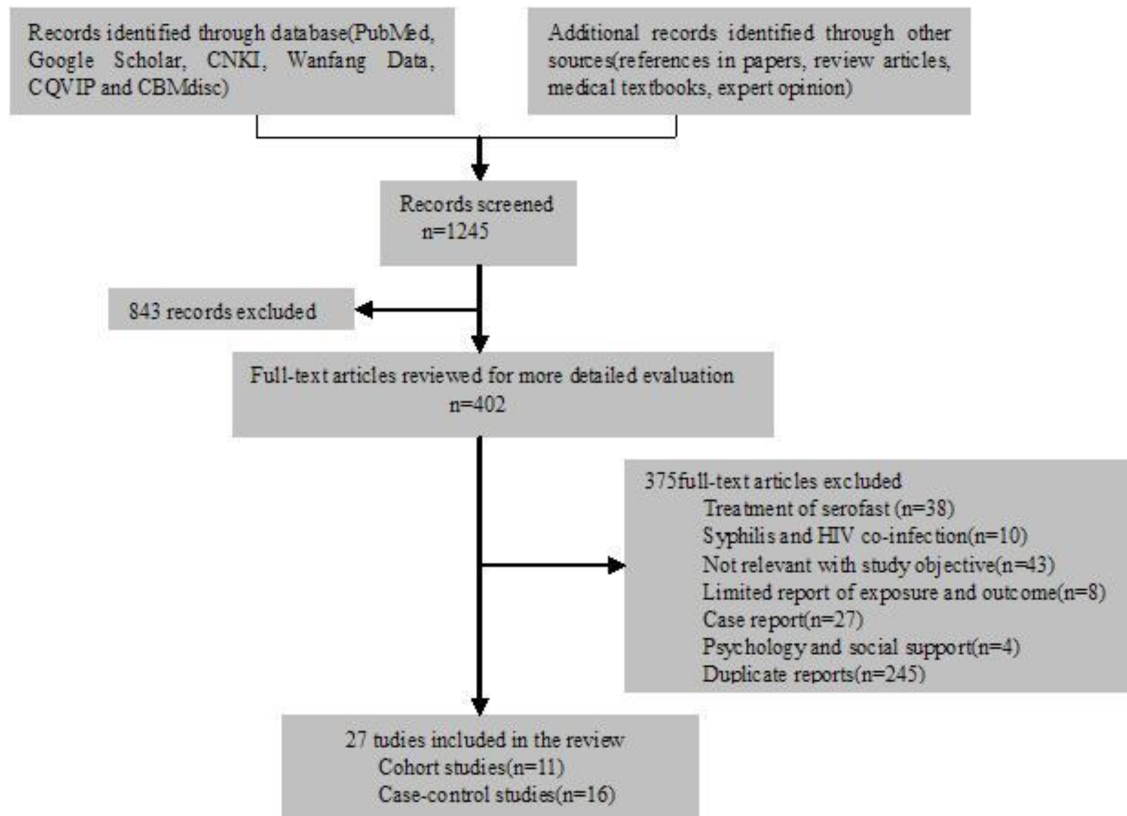

Fig. 1: Study identification flowchart

Table 1: Characteristics of 27 included studies

Reference number	Location	Study design/period	Duration of follow-up	Study population	Exposure	Outcomes	Methods of data collection	Comment
17	Guangzhou	Retrospective cohort/1994-2003	2 years	423 patients	Age, gender, stage of syphilis and initial titers of non-treponema antibodies	Serofast status and serological cure	Medical records	In this study, the clinical data of patients with syphilis in the recent ten years were reviewed, the incidence and the duration of occurrence of serofast, the relationship between serofast and age, sex, stage of disease, RPR initial titer of the patients were analyzed respectively by multiple regression
18	Beijing	Retrospective cohort/2001-2005	2 years	131 patients	Age, gender, initial titers of non-treponema antibodies, stage of syphilis and treatment	Serofast status and serological cure	Medical records	In this study, the incidence of serofast was investigated and the correlation between serofast and factors including age, gender, initial titer, disease course, and medications were analyzed.
25	Zhangzhou	Retrospective cohort/2002-2006	2 years	224 patients	Stage of syphilis	Serofast status and serological cure	Medical records	In this study, based on 5 years clinical data, the incidence of serofast was investigated and the correlation between serofast and stage of syphilis was analyzed.
26	Liuzhou	Retrospective cohort/2002-2006	2 years	318 patients	Stage of syphilis	Serofast status and serological cure	Medical records	In this study, the incidences of serofast based on stage of syphilis and the time when serofast were formed were analyzed.
27	Beijing	Retrospective cohort/unknown	Unknown	172 patients	Stage of syphilis, treatment, T-lymphocyte subset and NK cells in the peripheral blood	Serofast status and serological cure	Medical records and Flow cytometry	In this study, the correlation between serofast and related factors including initial titer of RPR, disease course, and medication. Flow cytometry was used to analyze T-lymphocyte subset and NK cells in the peripheral blood of untreated syphilitic patients, syphilitic patients whose serology turned negative after treatment, serofast patients, and healthy controls.
28	Guangzhou	Retrospective cohort/2005-2009	>2 years	366 patients	Stage of syphilis and treatment	Serofast status and serological cure	Medical records	In this study, the incidences of serofast based on stage of syphilis, treatment factors, and the time when serofast were formed were analyzed.
29	Guangzhou	Prospective cohort/2008-2009	1 years	102 patients	Subtypes of *Treponema pallidum* repeat(tpr) gene	Serofast status and serological cure	Medical records and Polymerase Chain Reaction(PCR)	In this study, specimens of confirmed patients with untreated early syphilis were collected by multiple centers cooperation. *Treponema pallidum* basic membrane protein (bmp) genes were screened from these specimens by nested PCR. After standard treatment, the relationship between the change of serum TRUST titer of the patients and the subtypes of tpr gene were analyzed.
30	Shanghai	Prospective cohort/ 2002-2009	>2years	420 patients	Stage of syphilis	Serofast status and serological cure	Medical records	In this study, the incidences of serofast based on stage of syphilis and the association between serofast and stage of syphilis were analyzed.
14	Xiamen	Prospective cohort/2005-2009	1 year	1308 patients	Stage of syphilis and treponema pallidum(TP) IgM antibody	Serofast status and serological cure	Medical records	In this study, TP-IgM was detected with FTA-Abs and TPPA. Syphilis patients were divided into experimental groups according to the results of TRUST and TPPA. Relationship between serofast, stage of syphilis, and TP-IgM were analyzed.
11	Beijing	Prospective cohort/2000-2010	>6 months	501 patients	Demographic characteristics, clinical characteristics, and behavior characteristics	Serofast status and serological cure	Medical records	In this study, analysis of factors determining the serological response to treatment was performed in HIV-negative patients with early syphilis, using demographic characteristics, clinical characteristics, and behavior characteristics
10	Xiamen	Prospective cohort/2005-2010	1 year	1327 patients	Age, gender, initial titers of non-treponema antibodies, and stage of syphilis	Serofast status and serological cure	Medical records	In this study, the incidence of serofast was investigated and the correlation between serofast and factors including age, gender, initial titer, and disease course were analyzed.

Abbreviation: PCR= polymerase chain reaction; FTA-Abs= fluorescent treponemal antibody absorption; TPPA= Treponema pallidum particle agglutination; TP= Treponema Pallidum

Table 1: Continued

Reference number	Location	Study design/period	Case group/sample size	Control group/sample size	Exposure	Measure of exposure	Comment
31	Guangzhou	HCC/unknown	**Serofast group/32 cases**	Healthy group/30 cases	T-lymphocyte subset and NK cells in the peripheral blood	Flow cytometry	In this study, the levels of T-lymphocyte subset and NK cells in the peripheral blood between serofast group and healthy group were compared.
32	Guangzhou	HCC/unknown	Serofast group/38 cases	Healthy group/23 cases	T-lymphocyte subset and NK cells in the peripheral blood	Flow cytometry	In this study, the levels of T-lymphocyte subset and NK cells in the peripheral blood between serofast group and healthy group were compared.
33	Shenzhen	HCC/unknown	Serofast group/30 cases	Healthy group/30 cases	T-lymphocyte subset	Flow cytometry	In this study, the levels of T-lymphocyte subset in the peripheral blood between serofast group and healthy group were compared.
34	Nanchang	HCC/2004-2006	Serofast group/20 cases	Healthy group/20 cases; Serological cure/20 cases	The levels of IFN-r and IL-10 in the serum	ELISA	In this study, the levels of IFN-r and IL-10 in the serum of serofast patients, syphilitic patients whose serology turned negative after treatment, and healthy controls were compared, respectively.
35	Guangzhou	HCC/2001-2006	Serofast group/58 cases	Serological cure/60 cases	T-lymphocyte subset in the peripheral blood	Flow cytometry	In this study, the levels of T-lymphocyte subset in the peripheral blood between serofast patients and syphilitic patients whose serology turned negative after treatment were compared.
36	2 cities [a]	HCC/2003-2006	Serofast group/38 cases	Healthy group/23 cases	The levels of IL-12 and IL-10 in the serum	ELISA	In this study, the levels of IL-12 and IL-10 in the serum of serofast patients and healthy group were compared.
37	Chengdu	HCC/2005-2007	Serofast group/20 cases	Healthy group/30 cases; Serological cure/30 cases	T-lymphocyte subset and NK cells in the peripheral blood	Flow cytometry	In this study, the levels of T-lymphocyte subset and NK cells in the peripheral blood of serofast patients, syphilitic patients whose serology turned negative after treatment, and healthy controls were compared.
38	Chongqing	HCC/2004-2006	Serofast group/20 cases	Healthy group/30 cases; Serological cure/20 cases	The levels of IL-2 and IL-10 in the serum	ELISA	In this study, the levels of IL-2 and IL-10 in the serum of serofast patients, syphilitic patients whose serology turned negative after treatment, and healthy controls were compared, respectively.
39	Suzhou	HCC/2008-2009	Serofast group/23 cases	Healthy group/20 cases; Serological cure/20 cases	T-lymphocyte subset and NK cells in the peripheral blood	Flow cytometry	In this study, the levels of T-lymphocyte subset and NK cells in the peripheral blood of serofast patients, syphilitic patients whose serology turned negative after treatment, and healthy controls were compared.
40	Hefei	HCC/2005-2009	Serofast group/25 cases	Healthy group/20 cases;	T-lymphocyte subset and NK cells in the peripheral blood	Flow cytometry	In this study, the levels of T-lymphocyte subset and NK cells in the peripheral blood of serofast patients and healthy controls were compared.
41	Dongguan	HCC/2009-2010	Serofast group/18 cases	Healthy group/18 cases;	CD4(+)CD25(+) regulatory T cells in the peripheral blood and IL-17 in the serum	Flow cytometry and ELISA	In this study, the levels of CD4(+)CD25(+) regulatory T cells in the peripheral blood and IL-17 in the serum of serofast patients, and healthy controls were compared.
42	Shenzhen	HCC/2010-2011	Serofast group/60 cases	Healthy group/63 cases; Serological cure/61 cases	T-lymphocyte subset in the peripheral blood, and IL-17 and IL-23 in the serum	Flow cytometry and ELISA	In this study, the levels of T-lymphocyte subset in the peripheral blood, and IL-17 and IL-23 in the serum of serofast patients, syphilitic patients whose serology turned negative after treatment, and healthy controls were compared.
43	Jieyang	HCC/unknown	Serofast group/112 cases	Healthy group/58 cases; Serological cure/155 cases	CD4(+)CD25(+) regulatory T cells in the peripheral blood and IL-10, IL-6 and IFN-r in the serum	Flow cytometry and ELISA	In this study, the levels of CD4(+)CD25(+) regulatory T cells in the peripheral blood and IL-0, IL-6 and IFN-r in the serum of serofast patients, syphilitic patients whose serology turned negative after treatment, and healthy controls were compared.
44	Nanning	HCC/2007-2011	Serofast group/32 cases	Healthy group/22 cases	The levels of IL-2, IL-4, IL-10, and IL-12 in the serum	ELISA	In this study, the levels of IL-2, IL-4, IL-10, and IL-12 in the serum of serofast patients, and healthy controls were compared.
45	Nanning	HCC/2007-2011	Serofast group/32 cases	Healthy group/11 cases	T-lymphocyte subset and NK cells in the peripheral blood	Flow cytometry	In this study, the levels of T-lymphocyte subset and NK cells in the peripheral blood of serofast patients, and healthy controls were compared.
46	Hanchuan	HCC/2007-2011	Serofast group/46 cases	Healthy group/22 cases	T-lymphocyte subset and NK cells in the peripheral blood	Flow cytometry	In this study, the levels of T-lymphocyte subset and NK cells in the peripheral blood of serofast patients, and healthy controls were compared.

Abbreviation: HCC=hospital-based case-control study; ELISA=enzyme linked immunosorbent assay/ [a] 2 cities include Zhangjiakou, and Guangzhou.

In two studies (11, 39), serological cure was defined as either negative nontreponemal antibody test results or a ≥4-fold (2 dilution) decrease in titer after treatment. In remaining studies, serological cure was defined as negative nontreponemal antibodies test results after treatment. For all included studies, serofast status was defined as either no change in titer or a ≤2-fold (1 dilution) titer decrease or increase from baseline, and the titer range at the diagnosis of serofast status was from 1:1 to 1:8. All included studies scored at least six stars according to NOS score system (Table 2), so they were considered of low-risk of bias.

Table 2: Star template of cohort and case-control studies based on NOS assessment

	Case-control star template						Cohort star template				
NO	**Selection**	**Comparability**	**Exposure**	**Total**	**Bias assessment**	**Reference number**	**Selection**	**Comparability**	**Outcome**	**Total**	**Bias assessment**
31	☆☆☆	☆	☆☆☆	☆☆☆☆☆☆☆	Low risk	**17**	☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk
32	☆☆☆	☆	☆☆☆	☆☆☆☆☆☆☆	Low risk	**18**	☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk
33	☆☆	☆	☆☆☆	☆☆☆☆☆☆	Low risk	**25**	☆☆	☆	☆☆☆	☆☆☆☆☆☆	Low risk
34	☆☆☆	☆	☆☆☆	☆☆☆☆☆☆☆	Low risk	**26**	☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆	Low risk
35	☆☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆☆	Low risk	**27**	☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk
36	☆☆	☆	☆☆☆	☆☆☆☆☆☆	Low risk	**28**	☆☆	☆	☆☆☆	☆☆☆☆☆☆	Low risk
37	☆☆☆☆	☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk	**29**	☆☆☆☆	☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk
38	☆☆	☆	☆☆☆	☆☆☆☆☆☆	Low risk	**30**	☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk
39	☆☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆☆	Low risk	**14**	☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk
40	☆☆☆	☆	☆☆	☆☆☆☆☆☆	Low risk	**11**	☆☆	☆	☆☆☆	☆☆☆☆☆☆	Low risk
41	☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk	**10**	☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk
42	☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk						
43	☆☆☆☆	☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk						
44	☆☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆☆	Low risk						
45	☆☆☆☆	☆☆	☆☆☆	☆☆☆☆☆☆☆☆☆	Low risk						
46	☆☆☆☆	☆	☆☆☆	☆☆☆☆☆☆☆☆	Low risk						

Clinical characteristics of patients with syphilis associated with serofast

Trend test showed syphilitic patients with an older age were prone to be serofast after treatment (P_{trend}=0.001). Female patients(sRR=1.50, 95%CI:1.34 to 1.68), latent syphilis ($sRR_{latent\ vs\ primary}$=3.17, 95%CI: 2.66 to 3.77; $sRR_{latent\ vs\ secondary}$=2.00, 95%CI: 1.48 to 2.69), and using non-penicillin treatment(sRR=2.99, 95%CI: 2.45 to 3.67) also increased the risk of serofast, but high baseline titers(>1:32) of nontreponemal antibodies(sRR=0.63, 95%CI: 0.54 to 0.75) decreased the risk of serofast (Table 3). There was no evidence of heterogeneity except for stage of syphilis (all *P* values ≤0.0003; I^2=71 to 84%). One study (29) showed the TP repeat gene subtype i increased the risk of serofast, when it was compared with subtype d (RR=4.67, 95%CI: 1.31 to 16.69) and subtype e (RR=5.00, 95%CI: 0.33 to 75.11), respectively. In one study(14), TP-IgM antibody between serofast patients and syphilitic patients whose serology turned negative after treatment(i.e., serological cure) was detected by fluorescent treponemal antibody absorption(FTA-Abs) and TPPA, and the results showed positive FTA-Abs-TP-IgM(RR=3.27, 95%CI: 2.89 to 3.69) and TPPA-TP-IgM(RR=3.26, 95%CI: 2.89 to 3.68) test increased the risk of serofast, which implied the serofast was associated with occult TP infection.

Table 3: Meta-analysis of association between clinical features of patients with syphilis an serofast status

Clinical features	Number of studies	Participants	Statistical methods	RR(95%CI)	Heterogeneity *Q*	*P*	I^2
Age(year)							
>=40 *vs* <=24	3	963	RR (M-H, FEM, 95% CI)	2.53(1.53,4.19)*	4.03	0.57	0%
>=40 *vs* 25-39	3	1606	RR (M-H, FEM, 95% CI)	1.99(1.32,3.01)*	3.29	0.73	0%
gender(female *vs* male)	7	3251	RR (M-H, FEM, 95% CI)	1.50(1.34,1.68)*	3.38	0.76	0%
initial titers of non-treponema antibodies (>1:32 *vs* <=1:32)	4	2552	RR (M-H, FEM, 95% CI)	0.63(0.54,0.75)*	0.67	0.71	0%
Stage of syphilis							
Latent *vs* primary	11	3103	RR (M-H, FEM, 95% CI)	3.17(2.66,3.77)*	15.64	0.11	36%
Latent *vs* secondary	10	3747	RR (M-H, REM, 95% CI)	2.00(1.48,2.69)*	55.51	<0.00001	84%
Secondary *vs* primary	10	3009	RR (M-H, REM, 95% CI)	1.71 (1.14,2.57)*	30.93	0.0003	71%
Treatment(non-penicillin *vs* penicillin)	3	669	RR (M-H, FEM, 95% CI)	2.99(2.45,3.67)*	0.72	0.70	0%
Subtypes of TP repeat gene							
Subtype i *vs* subtype d	1	36	RR (M-H, FEM, 95% CI)	4.67 (1.31,16.69)*	—	—	—
Subtype i *vs* subtype e	1	12	RR (M-H, FEM, 95% CI)	5.00 (0.33,75.11)	—	—	—
TP IgM antibody							
FTA-Abs(positive *vs* negative)	1	1208	RR (M-H, FEM, 95% CI)	3.27(2.89,3.69)*	—	—	—
TPPA(positive *vs* negative)	1	1208	RR (M-H, FEM, 95% CI)	3.26(2.89,3.68)*	—	—	—

Abbreviation:TP=treponema pallidum; FTA-Abs=fluorescent treponemal antibody absorption; TPPA= treponema pallidum particle agglutination
**Statistically significant (P<0.05)*

T-lymphocyte subset and NK cells in the peripheral blood associated with serofast

Present study showed that the serofast was associated with the levels of T-lymphocyte subset in the peripheral blood (Table 4). Compared with healthy group, the proportions of CD3(+)(SMD=-0.52, 95%CI: -0.71 to -0.34), CD4(+)(SMD=-0.67, 95%CI: -1.11 to -0.23), Th1 cells(SMD=-1.26, 95%CI: -2.00 to -0.52) and Th1/Th2(SMD=-1.52, 95%CI: -2.29 to -0.76) among serofast patients were decreased, but CD8(+)(SMD=0.58, 95%CI: 0.41 to 0.76), CD4(+)CD25(+) regulatory T cells(SMD=1.92, 95%CI: 1.57 to 2.26), and Th2 cells (SMD=0.91, 95%CI: 0.20 to 1.63) were increased. When compared with serological cure group, the CD4 (+) (SMD=-0.70, 95%CI: -1.26 to -0.14) among serofast patients was also decreased, but CD8 (+) (SMD=0.82, 95%CI: 0.09 to 1.56) and CD4 (+) CD25 (+) regulatory T cells (SMD=2.12, 95%CI: 1.82 to 2.42) were increased. When serological cure group was compared with healthy group, there were no significant difference for the levels of CD3 (SMD=-0.09, 95%CI: -0.38 to 0.20), CD4 (+)(SMD=-0.04, 95%CI: -0.33 to 0.24) and CD8(+) (SMD=-0.18, 95%CI: -0.66 to 0.30). Substantial heterogeneity was observed (all *P* values ≤ 0.006; I^2=57 to 94%).

Table 4: Meta-analysis of association between T-lymphocyte subset and NK cells in the peripheral blood and serofast status

T-lymphocyte subset and NK cells	Number of studies	Participants	Statistical methods	SMD(95%CI)	Heterogeneity *Q*	*P*	I^2
CD3(+)							
Serofast group *vs* healthy group	8	512	SMD (IV, REM, 95% CI)	-0.52(-0.71,-0.34)*	50.08	<0.00001	86%
Serofast group *vs* serological cure	4	332	SMD (IV, REM, 95% CI)	-0.58 (-1.21, 0.06)	22.03	<0.00001	86%
Serological cure *vs* healthy group	2	184	SMD (IV, FEM, 95% CI)	-0.09 (-0.38, 0.20)	0.17	0.68	0
CD4(+)							
Serofast group *vs* healthy group	9	555	SMD(IV, REM, 95% CI)	-0.67 (-1.11, -0.23)*	46.09	<0.00001	83%
Serofast group *vs* serological cure	4	332	SMD (IV, REM, 95% CI)	-0.70 (-1.26, -0.14)*	16.93	0.006	57%
Serological cure *vs* healthy group	2	184	SMD (IV, FEM, 95% CI)	-0.04 (-0.33, 0.24)	0.14	0.71	0
CD8(+)							
Serofast group *vs* healthy group	9	555	SMD(IV, FEM, 95% CI)	0.58(0.41, 0.76)*	12.31	0.14	35%
Serofast group *vs* serological cure	4	332	SMD (IV, REM, 95% CI)	0.82 (0.09, 1.56)*	28.09	<0.00001	89%
Serological cure *vs* healthy group	2	184	SMD(IV, REM, 95% CI)	-0.18 (-0.66, 0.30)	2.43	0.12	59%
CD4(+)/CD8(+)							
Serofast group *vs* healthy group	4	199	SMD (IV, REM, 95% CI)	-0.71 (-1.53, 0.10)	20.35	0.0001	85%
Serofast group *vs* serological cure	1	43	SMD(IV, FEM, 95% CI)	0.33(-0.28,0.93)	—	—	—
Serological cure *vs* healthy group	1	40	SMD(IV, FEM, 95% CI)	-1.89(-2.65,-1.13)*	—	—	—
CD4(+)CD25(+) regulatory T cells							
Serofast group *vs* healthy group	2	206	SMD (IV, FEM, 95% CI)	1.92 (1.57, 2.26)*	0.12	0.73	0%
Serofast group *vs* serological cure	1	267	SMD (IV, FEM, 95% CI)	2.12(1.82,2.42)*	—	—	—
NK cell							
Serofast group *vs* healthy group	7	372	SMD (IV, REM, 95% CI)	-0.85 (-1.80, 0.10)	97.40	<0.00001	94%
Serofast group *vs* serological cure	2	93	SMD (IV, FEM, 95% CI)	-0.13 (-0.54, 0.29)	0	1	0
Serological cure *vs* healthy group	1	60	SMD (IV, FEM, 95% CI)	0.48(-0.04,0.99)	—	—	—
Th1 cell							
Serofast group *vs* healthy group	1	43	SMD (IV, FEM, 95% CI)	-1.26(-2.00,-0.52)*	—	—	—
Th2 cell							
Serofast group *vs* healthy group	1	43	SMD (IV, FEM, 95% CI)	0.91(0.20,1.63)*	—	—	—
Th1/Th2							
Serofast group *vs* healthy group	1	43	SMD (IV, FEM, 95% CI)	-1.52(-2.29,-0.76)*	—	—	—

**Statistically significant (P<0.05)*

Interleukin and interferon in the serum associated with serofast

Finding from our study demonstrated that the serofast was associated with the levels of interleukin in the serum (Table 5). Compared with healthy group, the levels of IL-12 (SMD=-1.56 95%CI: -1.99 to -1.13), IL-2(SMD=-1.06, 95%CI: -1.66 to -0.45), IL-6(SMD= -1.09, 95%CI: -1.42 to -0.75), and IFN-r(SMD=-1.29, 95%CI: -2.31 to -0.26) among serofast patients was decreased, but IL-

10(SMD=1.68, 95%CI: 0.75 to 2.60) and IL-4(SMD=0.75, 95%CI: 0.19 to 1.32) were increased. When serofast patients were compared with serological cure group, the levels of IL-2(SMD=-1.03, 95%CI: -1.70 to -0.37) and IL-6(SMD=-1.00, 95%CI: -1.26 to -0.74) was also declined, but IL-10(SMD=2.23, 95%CI: 0.25 to 4.21) was elevated. For the levels of IL-10(SMD=0.11, 95%CI: -0.13 to 0.36), IL-2(SMD=-0.07, 95%CI: -0.63 to 0.50), IL-17(SMD=-0.01, 95%CI: -0.37 to 0.34), IL-23(SMD=-0.02, 95%CI: -0.37 to 0.33), IL-6(SMD=0.19, 95%CI: -0.11 to 0.50), and IFN-r(SMD=0.14, 95%CI: -0.14 to 0.41), there were no significantly statistical difference between serological cure group and healthy group. Substantial heterogeneity was found (all *P* values ≤ 0.006; I^2=87 to 97%).

Table 5: Meta-analysis of association between interleukin and interferon in the serum and serofast status

interleukin and interferon	Number of studies	Participants	Statistical methods	SMD(95%CI)	Heterogeneity *Q*	*P*	I^2
IL-10							
Serofast group *vs* healthy group	5	375	SMD(IV, REM, 95% CI)	1.68(0.75, 2.60)*	50.53	<0.00001	92%
Serofast group *vs* serological cure	3	347	SMD(IV, REM, 95% CI)	2.23 (0.25, 4.21)*	67.12	<0.00001	97%
Serological cure *vs* healthy group	3	303	SMD(IV, FEM, 95% CI)	0.11(-013, 0.36)	1.55	0.46	0
IL-17							
Serofast group *vs* healthy group	2	159	SMD (IV, FEM, 95% CI)	-0.15 (-0.46, 0.17)	1.72	0.19	42%
IL-12							
Serofast group *vs* healthy group	2	115	SMD (IV, FEM, 95% CI)	-1.56(-1.99, -1.13)*	1.63	0.20	39%
IL-2							
Serofast group *vs* healthy group	2	104	SMD (IV, FEM, 95% CI)	-1.06 (-1.66, -0.45)*	0.60	0.44	0
Serofast group *vs* serological cure	1	40	SMD (IV, FEM, 95% CI)	-1.03 (-1.70, -0.37)*	—	—	—
Serological cure *vs* healthy group	1	50	SMD (IV, FEM, 95% CI)	-0.07 (-0.63, 0.50)	—	—	—
IL-17							
Serofast group *vs* serological cure	1	121	SMD (IV, FEM, 95% CI)	-0.02 (-0.38, 0.33)	—	—	—
Serological cure *vs* healthy group	1	124	SMD (IV, FEM, 95% CI)	-0.01 (-0.37, 0.34)	—	—	—
IL-23							
Serofast group *vs* healthy group	1	123	SMD (IV, FEM, 95% CI)	0.14 (-0.21, 0.50)	—	—	—
Serofast group *vs* serological cure	1	121	SMD (IV, FEM, 95% CI)	-0.05 (-0.41, 0.30)	—	—	—
Serological cure *vs* healthy group	1	124	SMD (IV, FEM, 95% CI)	-0.02 (-0.37, 0.33)	—	—	—
IL-6							
Serofast group *vs* healthy group	1	170	SMD (IV, FEM, 95% CI)	-1.09(-1.42,-0.75)*	—	—	—
Serofast group *vs* serological cure	1	267	SMD (IV, FEM, 95% CI)	-1.00(-1.26,-0.74)*	—	—	—
Serological cure *vs* healthy group	1	213	SMD (IV, FEM, 95% CI)	0.19(-0.11,0.50)	—	—	—
IL-4							
Serofast group *vs* healthy group	1	54	SMD (IV, FEM, 95% CI)	0.75(0.19,1.32)*	—	—	—
IFN-r							
Serofast group *vs* healthy group	2	210	SMD (IV,REM, 95% CI)	-1.29 (-2.31, -0.26)*	7.41	0.006	87%
Serofast group *vs* serological cure	2	307	SMD (IV, REM, 95% CI)	-1.44 (-3.11, 0.23)	22.36	<0.00001	96%
Serological cure *vs* healthy group	2	253	SMD (IV, FEM, 95% CI)	0.14 (-0.14, 0.41)	2.0	0.16	50%

**Statistically significant (P<0.05)*

Publication bias

For gender(*Begg's test: Z=0.19, P=1; Egger's test: t=0.31, P=0.77; Macaskill's test: t=0.22,P=0.84*), latent syphilis *vs* primary syphilis(*Begg's test: Z=0.72,P=0.47; Egger's test: t=1.02,P=0.34; Macaskill's test: t=0.32,P=0.76*), latent syphilis vs secondary syphilis (*Begg's test: Z=0.31,P=0.76; Egger's test: t=1.44,P=0.12;Macaskill's test: t=1.02,P=0.21*), secondary syphilis vs primary syphilis(*Begg's test:Z=0.31,P=0.75;Egger's test: t=0.59, P=0.57;Macaskill's test: t=1.47, P=0.19*), CD3(+)(*Begg's test:Z=1.20,P=0.22;Egger's test:t=1.54,P=0.14;Macaskill's test:t=1.63,P=0.12*), CD4(+)(*Begg's test:Z=0.75,P=0.45; Egger's test:t=0.60,P=0.58; Macaskill's test:t=0.05,P=0.96*), CD8(+) (*Begg's test: Z=0,P=1; Egger's test:t=0.48,P=0.65; Macaskill's test:t=0.46,P=0.67*), and NK cells(*Begg's test: Z=0.75,P=0.45; Egger's test: t=0.59,P=0.57; Macaskill's test: t=1.02,P=0.21*), Egger's test, Begg's test, and Macaskill' test also indicated little evidence of publication bias(*all P>0.1*).

Sensitivity analysis

Sensitivity analyses were conducted to explore potential sources of heterogeneity in the association between serofast and potential risk factors and to examine the influence of various exclusion criteria on the overall risk estimate. In our study, sensitivity analysis was performed only for these factors including ≥6 studies such as gender, stage of syphilis, CD3 (+) (only for the comparison between serofast group and healthy group), CD4 (+)(only for the comparison between serofast group and healthy group), CD8 (+)(only for the comparison between serofast group and healthy group) and NK cells(only for the comparison between serofast group and healthy group). Exclusion of two studies (11, 39) in which serological cure was defined as either negative nontreponemal antibodies test or a ≥4-fold (2 dilution) decrease in titer after treatment, but in remaining studies, serological cure was defined as negative nontreponemal antibodies test after treatment and one study (11) in which the length from first treatment to diagnosis of serofast was more than 6 months but less than one year yielded similar results. Further exclusion of any single study did not materially alter the overall estimates.

Publication bias

Considering limited eligible studies for some potential predictors associated with serofast, we only aimed to these factors including ≥6 studies when we assessed publication bias. With regard to these factors, the Begg's funnel plot did not show any substantial asymmetry. In addition, Egger's test, Begg's test, and Macaskill' test also indicated little evidence of publication bias (all $P > 0.1$).

Discussion

The present study represents, to our knowledge, the first systematic evaluation to assess potential predictors that differentiate HIV-negative individuals from those without serological response by using a systematic review and meta-analysis. Our study including a large proportion of Chinese patients with syphilis (6682 HIV-negative participants infected with Syphilis) with sufficient statistical power aimed at addressing long-term clinical dilemma regarding the mechanism of serofast after treatment. Our findings may serve to guide clinicians regarding the identification of patients who may become serofast after therapy without clear evidence of treatment failure, and provide direct information for conducting targeted interventions in every community in the future, which will help to develop the optimal management program of serofast patients.

Presently, it is still not consistent for definition of the serofast (8-10, 16). Most Chinese experts think, it is reasonable that the length between first therapy and diagnosis of serofast is 12 months and 24 months for early syphilis(i.e., primary syphilis, secondary syphilis, and early latent syphilis) and late syphilis(infection time > 2 years), respectively(47). Some studies also indicated if patients' titers after treatment stay at some level (1:1 to 1:8) or have no change more than 3 months after treatment, it should be considered as serofast (47). In the present study, the shortest length from first treatment to diagnosis of serofast was more than 6 months, and the length in 92.3% of eligible studies was ≥ one year. For all of the studies, the titer range at the diagnosis of serofast was from 1:1 to 1:8.

Finding from our study showed that the serofast was positively associated with older age, female, latent syphilis as well as non-penicillin treatment, but negatively associated with higher baseline titers. Previous studies from other countries confirmed our results (9, 48, 49). Using non-penicillin, such as erythromycin and azithromycin, increased the risk of serofast, which implies that penicillin remains preferred drug of choice for treatment of syphilis. However, Sena AC (9) using data from a large, prospective, controlled trial showed the advantage that using benzathine penicillin to prevent serofast did not exist, when compared with azithromycin. It's worth noting that one study (29) hinted that the serofast was also positively associated with subtype i of TP repeat gene. However, in this study, the sample size is small. So the association between serofast and subtypes of TP repeat gene need further research. Previous studies (50, 51) also demonstrated that TP repeat gene family has strong variability, which allows TP to evade the body's immune destruction and leads to chronic infection of syphilis. In addition, one study also suggested that the serofast was positively associated with TP occult infection (15).

It seems that solely relying on clinical characteristics does not fully explain the mechanism of serofast. For example, previous studies(9) confirmed the relationship between the stage of infection and the baseline titer was evident in predicting treatment response, because participants with primary syphilis had a higher proportion of serological cure, whereas 43-58% of patients with secondary or early latent syphilis and baseline titers ≤1:32 were serofast at 6 months after treatment. Recently, investigators attempted to distinguish the cellular response in syphilitic patients after treatment by analyzing the levels of T-lymphocyte subset and NK cells in the peripheral blood, and Interleukin and interferon in the serum associated with serofast (27, 31-46). From the perspective of the body immune response, our study implies that the serofast was mainly associated with CD4(+) and CD8(+) cells levels in the peripheral blood, and IL-2, IL-6 and IL-10 levels in the serum, and specific performance: the levels of CD4(+), IL-2, and IL-6 are decreased, but the levels of CD8 and IL-10 are increased. Previous studies (50, 51) also suggested that serological response after treatment of syphilis was directly associated with suppression and disorders of body' immune function. However, some studies found no differences in cell types or proportions among individuals with serofast after treatment, compared with normal controls (27). So further investigations are essential to elucidate the biological basis for the serofast state and to determine whether serofast patients should undergo continued serological monitoring, retreatment, or cerebrospinal fluid examination for TP involvement.

Potential limitations of this study should be considered. Firstly, residual confounders are always of concern in observational studies. Although most included studies adjusted for a wide range of potential confounders for serofast, we still could not exclude the possibility that other unmeasured or inadequately measured factors have confounded the true association. Secondly, there are no widely accepted standards for diagnosis of serofast, and it is unclear for the optimal time point for assessment of serological response. Therefore, the random misclassification of serofast may influence the results. Thirdly, the results have to be viewed with caution because there was significant heterogeneity (I^2: from 57 to 97%). Although there was still evidence of heterogeneity, after sensitivity analyses, the result with very few changes was stable and reliable. In addition, the assessment also indicated little evidence of publication bias. So our estimates were considered reasonable. However, these estimates have to be viewed with caution because there was significant heterogeneity. Fourthly, our relatively strict inclusion criteria might have introduced selection bias. Finally, because the results of the current analysis were mainly based on data from Chinese populations, additional research in other populations is warranted to generalize the findings.

Conclusion

The present study represents, to our knowledge, the first systematic evaluation to assess potential predictors that differentiate HIV-negative individ-

uals from those without serological response, using a systematic review and meta-analysis. Although the role of potential bias and evidence of heterogeneity should be carefully evaluated, finding from our study indicated that the older age, female, lower baseline titers, latent course of infection, non-penicillin treatment, TP repeat gene subtype i, TP occult infection as well as the levels of CD4 (+), CD8 (+),IL-2, IL-6 and IL-10 among HIV-negative patients with syphilis should be considered as important predictors of serofast. However, until now the definition and mechanism of serofast has still been not clear, and it is necessary for further research.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

The authors want to thank the Fundamental Research Funds for Central South University (2012zzts029) and the Hunan Province Innovation Projects (CX2012B076) of China for their funding support. Authors also thank editors and reviewers for their suggestions. The authors declare that they have no conflicts of interest.

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