

## THE CLINICAL SIGNIFICANCE OF ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGENS IN PATIENTS WITH SYPHILIS

Zhou J<sup>1\*</sup>, Li Y<sup>2</sup>, Hu S<sup>1</sup>, Kong C<sup>3</sup>

<sup>1</sup>Medical Laboratory, Affiliated Hospital of Jining Medical University, Jining, P.R. China.

<sup>2</sup>Medical College, Jining Medical University, Jining, P.R. China.

<sup>3</sup>Nursing Department, Affiliated Hospital of Jining Medical University, Jining, P.R. China.

\*Correspondence: Jianwei Zhou; +86-537-2903218; immunolife@126.com

### Abstract

**Background:** To date, the pathogenic mechanism of *Treponema pallidum* (TP), the pathogen that causes syphilis remains unclear. Recently, some scholars found that the progression of syphilis infection probably relates to the production of autoantibodies stimulated by TP.

**Objective:** To determine and compare the presence of serum antibodies to extractable nuclear antigens (ENAs-Ab) between patients with syphilis and healthy controls who do not have syphilis.

**Materials and Method:** This study was a descriptive comparative study carried out between January 2014 and December 2015 at the Affiliated Hospital of Jining Medical University, Jining, P.R. China; 220 patients with *Treponema pallidum* particle agglutination assay (TPPA) - confirmed syphilis were included and another 220 healthy persons who were TPPA-negative were included as the controls. Presence of ENAs-Ab (detected by immunoblotting method) were compared in both groups. Differences in presence of ENAs-Ab in patients with syphilis by gender, age and Tolidine Red Unheated Serum Test (TRUST) result was also determined. Statistical significance was pValue <0.05.

**Results:** Among the patients with syphilis, antibodies to Sjögren's Syndrome antigen A (SSA), Ro/SSA antigen of 52 000 Molecular Weight (Ro-52), double stranded deoxyribonucleic acid (dsDNA), proliferating cell nuclear antigen (PCNA), Ribosomal P protein (Po) and Sjögren's Syndrome antigen B (SSB) were present in 10.9%, 16.8%, 4.0%, 7.2%, 3.2% and 7.7% respectively. Significantly higher number of patients with syphilis had ENAs-Ab compared to healthy controls (P<0.05). There was no significant difference in the number of TRUST-positive and TRUST-negative patients who had ENAs-Ab (P>0.05). Significantly more females than males with syphilis had antibodies to SSA, Ro-52 and SSB (P<0.05). The 4 most common ENAs-Ab detected were found more in patients over 45 years with syphilis.

**Conclusion:** Syphilis infection appears to trigger an autoimmune response and ENAs-Ab may be a suitable clinical index to monitor progression of infection.

**Keywords:** *Treponema pallidum*, Autoantibody, Extractable nuclear antigens, Tolidine Red Unheated Serum Test.

**Cite this article:** Zhou J, Li Y, Hu S, Kong C. The clinical significance of antibodies to extractable nuclear antigens in patients with syphilis. Yen Med J. 2021;3(2):135–138.

### INTRODUCTION

Syphilis is a chronic and systemic infectious disease caused by *Treponema pallidum* (TP) and can be transmitted between persons through sexual intercourse, mother-to-child-transmission (transplacental) and via blood transfusion. *Treponema pallidum* can invade every tissue type in the body including the skin, eyes, bones, nerves etc. Syphilis thus has various clinical manifestations and may have serious health implications. To date, the pathogenic mechanism of TP remains

unclear. Recently, some scholars found that the progression of syphilis infection probably relates to the production of autoantibodies stimulated by TP.<sup>1,2</sup> This study aimed to explore the clinical significance of antibodies to extractable nuclear antigens (ENAs-Ab) in patients with syphilis by determining and comparing the presence of serum ENAs-Ab between patients with syphilis and healthy controls who do not have syphilis, and by describing characteristics of patients with syphilis who have ENAs-Ab.

## MATERIALS AND METHODS

This was a descriptive comparative study carried out between January 2014 and December 2015 at the Affiliated Hospital of Jining Medical University, Jining, P.R. China. Participants in this study were recruited from among the patients visiting the hospital medical laboratory for serological tests and from among healthy individuals who had come for medical check-up in the hospital. The cases were 220 patients with Treponema pallidum particle agglutination assay (TPPA)-confirmed syphilis, while another 220 healthy persons who were TPPA-negative were included as the controls. Hepatitis B virus, hepatitis C, human immunodeficiency virus, tumours, use of immune-suppressive drugs and use of any enhancement drugs in the previous six months were ruled out among the participants in both groups. Only participants who gave an informed consent were included in the study.

The TPPA kit used for this study was sourced from Mp Biomedical Asia Pacific Pte. Ltd., Singapore; TRUST kit sourced from Shanghai Rongsheng Biological Pharmaceutical Co., Ltd., Shanghai, China; ENA antibody kit sourced from HOB Biological Pharmaceutical Co., Ltd., Jiangsu, China; Blotrary-866 Automatic Protein Detection device sourced from Rayto Life and Analytical Sciences Co., Ltd, Shenzhen, China.

Two millilitres of venous blood was drawn from each of the participants and centrifuged at 2000 revolutions per minute for 10 minutes. The sera obtained were used to detect antibodies to ENAs. TRUST was also performed according to the manufacturer's operational instructions.

Ethical approval for the study was obtained from the research ethics committee of the Affiliated Hospital of Jining Medical University, Jining, P.R. China. Data from this study were analyzed using SPSS Statistics version 19.0 statistical software. The association between categorical variables were tested using the Pearson's chi-square test and statistical significance was a p-value < 0.05.

## RESULTS

There were 164 males and 56 females with syphilis while the healthy control group had 160 males and 60 females. The patients with syphilis were between age 26 years and 72 years with a mean age of 44±12 years, while the

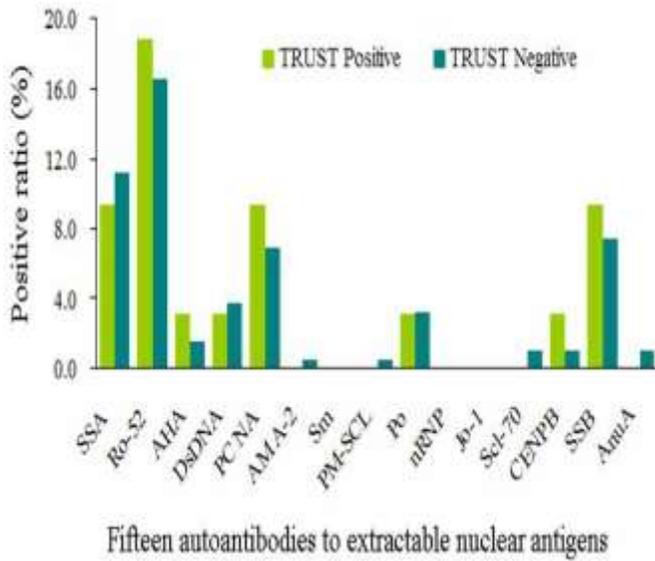
healthy participants were between age 23 years and 68 years and the mean age was 50±9 years. As shown in Table 1, among the patients with syphilis, antibodies to Sjögren's Syndrome antigen A (SSA), Ro/SSA antigen of 52 000 Molecular Weight (Ro-52), double stranded deoxyribonucleic acid (dsDNA), proliferating cell nuclear antigen (PCNA), Ribosomal P protein (Po) and Sjögren's Syndrome antigen B (SSB) were present in 10.9% (n = 24), 16.8% (n = 37), 4.0% (n = 8), 7.2% (n = 16), 3.2% (n = 7) and 7.7% (n = 17) respectively. Among the healthy controls, significantly fewer individuals had antibodies to SSA, Ro-52, dsDNA, PCNA, Po and SSB; 2.3%, 4.1%, 0.9%, 2.7%, 0% and 0.9% respectively when compared to the patients with syphilis, p < 0.05.

**Table 1: Presence of ENAs-Ab in patients with syphilis and health controls**

ENAs-Ab	Patients with syphilis		Health controls	
	Positive	Positive (%)	Positive	Positive (%)
anti-SSA	24*	10.9	5	2.3
anti-Ro-52	37*	16.8	9	4.1
anti-AHA	4	1.8	1	0.5
anti-DsDNA	8*	4.0	2	0.9
anti-PC NA	16*	7.2	6	2.7
anti-AM A-2	1	0.5	0	0
anti-Sm	0	0	0	0
anti-PM-SCL	1	0.5	0	0
anti-Po	7*	3.2	0	0
anti-nRNP	0	0	0	0
anti-Jo-1	0	0	0	0
anti-Scl-70	2	0.9	0	0
anti-CENPB	3	13.6	0	0
anti-SSB	17*	7.7	2	0.9
anti-AnuA	2	0.9	0	0

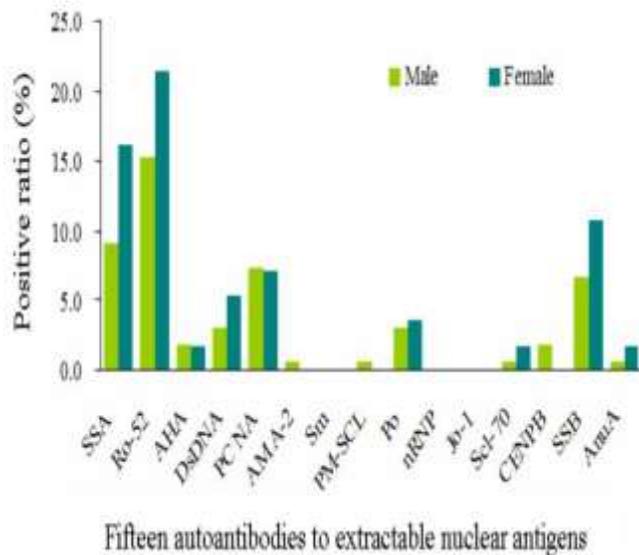
\*Statistically significant (p<0.05)

Among the patients with TPPA-confirmed syphilis, there were 32 persons who were also TRUST-positive and there was no significant difference in the number of TRUST-positive and TRUST-negative patients who had ENAs-Ab (P>0.05). Figure 1 shows how the presence of ENAs-Ab varied between TRUST-positive and TRUST-negative patients.

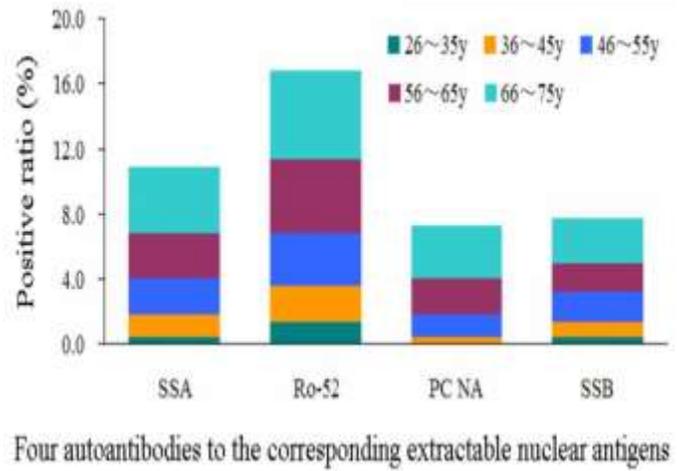


**Figure 1: Presence of ENAs-Ab compared between TRUST-positive and TRUST-negative patients with syphilis**

Significantly more females than males with syphilis had antibodies to SSA, Ro-52 and SSB (P<0.05). Figure 2 shows how the presence of ENAs-Ab is distributed between male and female participants. As shown in Figure 3, the 4 most common ENAs-Ab detected were found more in patients over 45 years with syphilis.



**Figure 2: Distribution of ENAs-Ab between male and female participants**



**Figure 3: Distribution of antibodies to SSA, Ro-52, PCNA and SSB among the different age-groups of patients with syphilis.**

**DISCUSSION**

According to the studies on humoral and cellular immunity, it has been proven that patients suffer immune-mediated injury following TP infection.<sup>3,4</sup> However, the immunological mechanism remained unclear. In this study, the clinical significance of antibodies to extractable nuclear antigens (ENAs-Ab) in patients with syphilis was explored by determining and comparing the presence of serum ENAs-Ab between patients with syphilis and healthy controls who do not have syphilis, and by describing characteristics of patients with syphilis who have ENAs-Ab. As seen in the results from this study, antibodies to SSA, Ro-52, dsDNA, PCNA, Po and SSB were present in a significantly higher number of patients with syphilis when compared with healthy controls. These finding is consistent with that from other studies which reported that ANA were found more in patients with syphilis than in healthy individuals,<sup>1,2</sup> and the result of this study with that of the other cited studies strongly suggests that TP infection could cause autoimmune response which probably underlies immune-mediated injury. As for the mechanism of such injury, it is probably due to the autoimmune response stimulated by the autoantigens which are released from the organs invaded by TP, and this could be detected with antoantibodies.<sup>5,6</sup>

TRUST is a common and non-specific index for diagnosis of TP infection, used mainly to determine the clinical stages and observe therapeutic efficacy. In this study, there was no significant difference in the number of TRUST-positive and TRUST-negative patients who had ENAs-Ab. This probably

implies that there was no correlation between TRUST results and the generation of autoantibodies. The possible reason for the irrelevance is that syphilis is a chronic progressive disease in which immune response follows persistent stimulation by TP, while TRUST becomes positive mainly at the early stage or the second phase of syphilis. Therefore, there is no correlation between TRUST-positive status and presence of antibodies to ENAs. However, in Feng's study,<sup>7</sup> the presence of ANA in TRUST-positive patients was significantly higher than in those who were TRUST-negative. The reasons for such variation between findings from these studies are probably attributable to the difference in the method used for detection of autoantibodies or in the sample size used. However, further studies in this area may give specific reasons for such differences.

The presence of ENAs-Ab in significantly more females than males in this study suggests that females are probably more vulnerable to immune-mediated injury from TP infection compared to the males. From this study, the 4 most common ENAs-Ab detected were found more in patients over 45 years with syphilis. This finding was consistent with a previous study which showed that ANA was present significantly more in older patients with syphilis than the younger population.<sup>8</sup> The reason for this phenomenon probably is that there may be an underlying immune disorder in older people. Under the stimulation of TP, autoantibodies, heterophile antibody, alpha-fetoprotein and other abnormal substances are generated and aggravates autoimmune response.<sup>9,10</sup>

In conclusion, this study shows that antibodies to ENAs in patients with syphilis are significantly higher than those of the healthy population. The presence of ENAs-Ab is relative to age and gender. Older and female patients are more vulnerable to immune-mediated injury following TP infection. In our opinion, detection of antibodies to ENA will be helpful to estimate, prevent and treat the immune injury in patients with syphilis.

#### CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest between them.

#### ACKNOWLEDGEMENT

This work is supported by the Research Fund for Lin He's Academician Workstation of New Medicine and Clinical Translation in Jining Medical University (JYHL2018FMS08), and the Project of scientific research

support fund for teachers of Jining Medical University (JYFC2018FKJ023).

#### REFERENCES

1. Wu SH, Zhou HQ, Qiu XJ. The clinical manifestation of anticardiolipin antibody and autoimmune antibody in the population with syphilis. *China Nat Med Fro.* 2013;8(4):82-83.
2. Zhu HW, Zhou WM, Guo Q, Yang Y. Analysis of sera antinuclear antibody and anticardiolipin antibody of 500 patients with syphilis. *Lab Med Clin.* 2013;10(8):2410-2411.
3. Liu SQ, Wang SP, Wu YM, Zhao FJ, Zeng TB, Zhang YJ, et al. Production of proinflammatory cytokines through induction of the human monocytic cell THP-1 by Tp2751 recombinant protein of *Treponema pallidum*. *Sci China Life Sci.* 2010;40:48-54.
4. Brinkman MB, Mc Gill MA, Ptersson J, Rogers A, Matejková P, Smajs D, et al. A novel *Treponema pallidum* antigen, TP0316, is an outer membrane protein that binds human fibronectin. *Infect Immun.* 2008;76(5):1848-1857.
5. Zhang HP, Ren RX, Lai DH, Lian S. Anticardiolipin antibody in diagnosing syphilis. *Chin J Dermatol.* 2013;27(5):482-484.
6. Ma CJ, Lin Y, Xia WY, Ni F. Analysis of sera anticardiolipin antibody of the patients with infection of *Treponema pallidum*. *Int J Lab Med.* 2012;33(17):2150-2151.
7. Feng JK, Ma DY, Jiang ZD, Chen SF, Chen J, Jin WW. Detection and significance of sera antinuclear antibody of the patients with syphilis. *Contemp Med.* 2011;17(33):5-6.
8. Cai SJ, Zhou JW, Yang YJ, Zhang ZC, Kong C, Shen AH. Detection of serum antinuclear antibody of the elderly patients with syphilis and its clinical significance. *Chin J Health Lab Technol.* 2016;26(1):64-65.
9. Augenbraun M, French A, Glesby M, Sanchez-Keeland L, Young M, Greenblatt R, et al. Hepatitis C virus infection and biological false positive syphilis tests. *Sex Transm Infect.* 2010;86(2):97-98.
10. Men K, Wei DJ, Li LY, Cheng Y. Analysis of antinuclear antibody and ENA antibody detection results in 286 healthy veteran cadres in Tianjin. *Chin J Health Lab Technol.* 2014;24(1):114-116.