Antinuclear antibodies in scrub typhus: Transient occurrence during acute illness

Maria Koshy1, John Mathew2, Reginald Alex1, John Antony Jude3, Ravikar Ralph1, Thambu David Sudarsanam1, Sowmya Sathyendra1, J. Visalakshi4 & John Victor Peter5

1Department of Medicine; 2Department of Rheumatology; 3Department of Microbiology; 4Department of Biostatistics, 5Department of Critical Care, Christian Medical College & Vellore, India

ABSTRACT

Background & objectives: The pathological hallmark of scrub typhus infection is focal or disseminated vasculitis. As with other infections, antinuclear antibodies (ANA) have been previously described in scrub typhus. However, the underlying mechanisms and implications of this immunological phenomenon is not well understood. In the present work it was assessed whether ANA is associated with illness severity and outcomes.

Methods: In this prospective study spanning one year, patients fulfilling the diagnostic criteria for scrub typhus were recruited. Patients with other acute infective febrile illnesses were taken as controls. ANA positivity was compared between the cases and controls. ANA in scrub typhus was assessed for correlation with disease severity, organ dysfunction and outcomes.

Results: The cohort comprised of 149 patients (scrub 89; controls 60) with mean age 46.5 (SD=16.9) yr; 48.3% were female. ANA was detected in 48 (53.9%) patients with scrub typhus and 9(15%) controls (p < 0.001). The ANA pattern was predominantly speckled (93.8%) in both scrub typhus patients and controls. In patients with scrub typhus, ANA positivity was associated with increasing APACHE-III score [Odds ratio (OR) 1.01; 95% CI 0.99–1.03; p = 0.09]. On bivariate analysis, ANA tended to be correlated with acute respiratory distress syndrome (OR 2.32; 95% CI 0.98–5.46; p = 0.06), hepatic dysfunction (OR 2.25; 95% CI 0.94–5.39, p = 0.06) and aseptic meningitis (OR 6.83; 95% CI 0.80–58.05, p = 0.08). The presence of these antibodies did not correlate with duration of hospitalization or mortality. Convalescent sera on 31 ANA positive scrub typhus patients demonstrated persistence of ANA in only 5 (16.1%) patients.

Interpretation & conclusion: The disappearance of ANA during the convalescent phase suggests that ANA is expressed during the acute phase of scrub typhus infection. Its association with organ dysfunction warrants further study of the mechanisms and impact of autoantibody formation in scrub typhus.

Key words Immunology; rickettsial diseases; scrub typhus; tropical medicine
scrub typhus infection. We also assessed if the presence of ANA correlated with illness severity and outcomes.

MATERIAL & METHODS

Study design

This prospective observational study was conducted in the medical wards and medical ICU of Christian Medical College, Vellore, a tertiary care referral teaching hospital in India, for a period of one year from January to December 2013.

All patients aged 16 year and above, who presented with an acute febrile illness of up to 21 days duration (documented temperature >100.4 °F), who fulfilled diagnostic criteria for scrub typhus were considered for inclusion as cases. Scrub typhus was defined as an acute febrile illness with (a) presence of an eschar and positive scrub IgM ELISA, or (b) a positive scrub IgM ELISA and defervescence within 48 h of initiation of doxycycline or azithromycin. Exclusion criteria were immuno-compromised state (HIV infection, pre-existing immuno-suppressive medication), autoimmune disease, malignancy and use of medication known to induce ANAs (e.g. sulfasalazine, praziquantel, isoniazid, hydralazine). As ANAs are also known to occur non-specifically with other acute infective febrile illnesses, controls were included for the study. Controls were patients with acute infective febrile illness, not fulfilling diagnostic criteria for scrub typhus. Every attempt was made to recruit age-sex matched controls admitted around the same time as a case; however, this was not always possible.

All patients were recruited within 24 h of admission and demographic details and laboratory data were documented. Acute physiology and chronic health evaluation III (APACHE-III) score was calculated on the day of admission and sequential organ failure assessment (SOFA) scores were calculated on Days 1, 3 and 5. Scrub typhus detect IgM ELISA (InBios International Inc., Seattle, WA, USA) was used for the serological diagnosis of scrub typhus; as per the manufacturer’s instructions an optical density of ≥0.5 was considered to be diagnostic of scrub typhus; as per the manufacturer’s instructions an optical density of ≥0.5 was considered to be diagnostic of scrub typhus; as per the manufacturer’s instructions an optical density of ≥0.5 was considered to be diagnostic of scrub typhus; as per the manufacturer’s instructions an optical density of ≥0.5 was considered to be diagnostic of scrub typhus; as per the manufacturer’s instructions an optical density of ≥0.5 was considered to be diagnostic of scrub typhus.
sis. Associations were expressed as odds ratio (OR) with 95% confidence intervals (CI). SPSS v20 for windows (IBM Corp., NY) was used for statistical analysis.

**Ethical statements**

The study was approved by the Institutional Review Board and ethics committee (IRB Approval no. 8158). Written and verbal informed consent was obtained from the study patients.

**RESULTS**

The cohort comprised of 149 patients (scrub 89; controls 60) with mean age 46.5 (SD = 16.9) yr; and 48.3% were females. The majority of the patients (89.9%) were from within a 50 km radius from the hospital. The mean duration of fever prior to presentation was 7.7 (95% CI; 7.1–8.5) days. An eschar was noted in 75.3% (n = 67) of the cases. Demographic data, treatment and outcome of patients with scrub typhus (cases) are summarized in Table 1. The control group comprised of patients with urinary tract infection (n = 22), liver abscess (n = 9), pneumonia (n = 9), dengue (n = 8), malaria (n = 6), enteric fever (n = 3), pyogenic meningitis (n = 2) and cellulitis (n = 1). When cases were compared with controls, although the mean age was similar, there were significantly more females in cases than controls (Table 2). Cases were sicker than controls with a significantly larger proportion of cases requiring mechanical ventilation (Table 2).

Antinuclear antibodies data is summarized in Table 2. Of the 89 patients with scrub typhus, ANA was detected in 48 (53.9%) patients. In contrast, in controls with other acute febrile illnesses, only 9 (15%) patients were ANA positive ($p < 0.001$). The ANA pattern was universally (100%) speckled in controls, while in those with scrub typhus, a majority (n = 45; 93.8%) had a speckled pattern and 3 (6.3%) had a nucleolar pattern of ANAs.

In patients with scrub typhus, acute respiratory distress syndrome (57.3%) and renal dysfunction (36%) were frequent, while complications such as aseptic meningitis (9%) and myocarditis (7.9%) were less commonly observed. Need for ventilation and vasoactive agents was in 26 (29.2%) and 20 (22.4%) patients respectively. Mortality was 3.3% (n = 3). In the control group, two patients required ventilation (3.3%). The duration of hospital stay and mortality in controls was similar to patients with scrub typhus (Table 2).

Repeat ANA was done during the convalescent phase

### Table 1. Demographics, treatment and outcomes of patients with scrub typhus

| Parameters | All patients (n = 89) | ANA positive (n = 48) | ANA negative (n = 41) | p-value* |
|------------|----------------------|----------------------|-----------------------|---------|
| Age (yr)   | 46.5 (16.9)          | 47.5 (16.1)          | 45.2 (17.9)           | 0.51    |
| Gender, Male : Female | 38 : 51 | 23 : 25 | 15 : 26 | 0.28 |
| Profession |                      |                      |                       |         |
| Professional/Skilled | 7 | 5 | 2 | 0.66 |
| Unskilled | 32 | 16 | 16 |       |
| Unemployed | 50 | 27 | 23 |       |
| Duration of symptoms (Days)† | 7 (5–10) | 7 (5–10) | 7 (7–10) | 0.70 |
| Eschar (n) | 67 (75.2%) | 36 (75%) | 31 (75.6%) | 0.94 |
| Admission | SOFA score | 5.6 (3.6) | 5.9 (3.7) | 5.3 (3.5) | 0.43 |
|           | APACHE III score     | 42.4 (24.3)          | 46.5 (28.4)           | 37.6 (17.6) | 0.23 |
| Complications (n) | Shock | 25 | 14 | 11 | 0.80 |
|               | ARDS                | 51                   | 32                    | 19       | 0.05 |
|               | Myocarditis         | 7                    | 5                     | 2        | 0.33 |
|               | Renal failure       | 32                   | 17                    | 15       | 0.90 |
|               | Aseptic meningitis  | 8                    | 7                     | 1        | 0.04 |
|               | Number ventilated (%) | 26 (29.2%) | 14 (29.1%) | 12 (29.2%) | 0.99 |
|               | Use of vasoactive agents (n) | 20 (22.4%) | 10 (20.8%) | 10 (24.3%) | 0.68 |
|               | Admission to ICU (n) | 17 (19.1%) | 7 (14.5%) | 10 (24.3%) | 0.24 |
|               | Hospital length of stay (Days)† | 6 (5–8) | 6 (5–8) | 6 (4.5–9) | 0.29 |
|               | Hospital mortality (n) | 3 (3.3%) | 2 (4.1%) | 1 (2.4%) | 0.65 |

*p-value comparisons are between anti-nuclear antibody (ANA) positive and ANA negative patients using chi-square test; †Median, inter-quartile range; p-value was calculated by comparing means using chi-square and t-test; SOFA–Sequential organ failure assessment; APACHE–Acute physiology and chronic health evaluation; ARDS–Acute respiratory distress syndrome; (n) indicates number of patients; Values in parentheses indicate standard deviation unless specified.
(around 6 wk) in 31 out of the 46 survivors (67.3%) with scrub typhus who were tested positive for ANA during the acute illness. Only 5 (16.1%) patients persisted to have ANAs and even in these patients there was a decrease in the intensity of ANA positivity on immunofluorescence.

Logistic regression analysis (Table 3) of ANA with organ dysfunction, illness severity and outcomes showed that SOFA scores did not correlate with ANA positivity on Day 1 (OR 1.04; 95% CI 0.93–1.17; \( p = 0.42 \)), Day 3 (OR 1.06; 95% CI 0.94–1.20; \( p = 0.32 \)) or Day 5 (OR 1; 95% CI 0.85–1.18; \( p = 0.93 \)). However, a statistically insignificant positive association between ANA positivity and APACHE-III score was found (OR 1.01; 95% CI 0.99–1.03; \( p = 0.09 \)).

Antinuclear antibodies positivity tended (\( p = 0.06 \)) to be correlated, with the development of acute respiratory distress syndrome (OR 2.32; 95% CI 0.98–5.46) and hepatic dysfunction (OR 2.25; 95% CI 0.94–5.39); this association was however weak (\( p = 0.08 \)) for aseptic meningitis (OR 6.83; 95% CI 0.80–58.05). It did not correlate with length of intensive care (\( p = 0.17 \)), hospital stay (\( p = 0.29 \)) or mortality (\( p = 0.65 \)).

**DISCUSSION**

This study explored the prevalence of ANA in scrub typhus infection and assessed its relationship with disease outcomes. A significantly (\( p < 0.001 \)) higher prevalence of ANA was found in scrub typhus patients (53.9%) in comparison to patients with other febrile illnesses (15%). These antibodies appear to develop rapidly, occurring in about a week following onset of fever. During the convalescent phase, only 16.1% of the scrub typhus patients assessed, continued to be ANA positive, suggesting that ANA positivity was a transient occurrence during acute illness. The prevalence of ANA positivity during the convalescent phase of illness was similar to that reported in the general population\(^6\) of 5–15%. Although, ANA positivity was not strongly correlated with illness severity at admission, it was associated, albeit weakly, with acute respiratory distress syndrome, hepatic dysfunction and aseptic meningitis. ANA positivity did not impact mortality or length of stay. The findings of this study suggest a possible association between these nonspecific auto-antibodies and the severity of illness, the significance of which is unclear and needs to be evaluated further.

The prevalence of ANA in infections such as malaria, tuberculosis and hepatitis B is well known and attributed to be an epiphenomenon\(^1^3\). The high prevalence of such antibodies in scrub typhus is not surprising since the pathogenesis is that of an infectious systemic vasculitis. The principle site of involvement in scrub typhus is the endothelial cell which results in a vasculopathy and increased microvascular permeability. This may account for the protean manifestations of this

---

**Table 2. Comparison of characteristics of scrub typhus patients with controls with acute febrile illnesses**

| Parameters                  | Scrub typhus (n = 89) | Controls (n = 60)* | p-value** |
|-----------------------------|-----------------------|-------------------|-----------|
| Age (yr)                    | 46.5 (16.9)           | 46.6 (18)         | 0.48      |
| Gender Male : Female        | 38 : 51               | 39 : 21           | 0.01      |
| ANA Positive (n)            | 48 (53.9%)            | 9 (15%)           | <0.001    |
| Negative (n)                | 41 (46%)              | 51 (85%)          | <0.001    |
| ANA titer (–)ve or weakly (+)ve | 41                      | 51                  |           |
| 1+                          | 23                    | 3                  |           |
| 2+                          | 22                    | 6                  |           |
| 3+                          | 3                     | 0                  |           |
| ANA pattern Speckled        | 45 (93.8%)            | 15 (100%)         | 1         |
| Speckled and nucleolar      | 3 (6.2%)              | 0                  |           |
| Admission SOFA             | 5.6 (3.6)             | 3.24 (2.5)         | <0.001    |
| Ventilation (n)            | 26 (29.2%)            | 2 (3.3%)          | <0.001    |
| Duration of stay            | 6 (5–8)               | 8 (5.7–13)        | 0.016     |
| Mortality                   | 3 (3.3%)              | 1 (1.7%)          | 0.45      |

*Controls were patients with acute infective febrile illness other than scrub typhus; **p-value comparisons between patients and controls using chi-square test; \(^{1}\)Median, interquartile range; \(^{2}\)Data available for 58 patients; ANA–Anti-nuclear antibody; SOFA–Sequential organ failure assessment; (n) indicates number of patients; Values in parentheses indicate standard deviation unless specified.

**Table 3. Bivariate analysis of antinuclear antibody (ANA) positivity and its association with severity of illness scores, organ dysfunction and outcomes**

| Parameters                  | Odds ratio | 95% Confidence interval | p-value |
|-----------------------------|------------|-------------------------|---------|
| Age (yr)                    | 1          | 0.98–1.03               | 0.50    |
| Scoring systems             |            |                         |         |
| APACHE III                  | 1.01       | 0.99–1.03               | 0.09    |
| SOFA (Day 1)                | 1.04       | 0.93–1.17               | 0.42    |
| MODS                        | 1.25       | 0.92–1.69               | 0.14    |
| Organ dysfunction           |            |                         |         |
| Myocarditis                 | 2.27       | 0.43–12.36              | 0.34    |
| Renal failure               | 0.95       | 0.40–2.26               | 0.91    |
| Circulatory shock           | 1.12       | 0.44–2.85               | 0.81    |
| ARDS                        | 2.32       | 0.98–5.46               | 0.06    |
| Aseptic meningitis          | 6.83       | 0.80–58.05              | 0.08    |
| Hepatic dysfunction         | 2.25       | 0.94–5.39               | 0.06    |
| Outcomes                    |            |                         |         |
| Duration of ICU stay        | 0.87       | 0.71–1.06               | 0.17    |
| Duration of hospital stay   | 0.94       | 0.85–1.04               | 0.29    |
| Mortality                   | 0.57       | 0.05–6.5                | 0.65    |

APACHE–Acute physiology and chronic health evaluation; SOFA–Sequential organ failure assessment; MODS–Multiple organ dysfunction score; ARDS–Acute respiratory distress syndrome.
disease, ranging from skin to central nervous system involvement. Inflammation is mediated via cytokines with a Th1 response and formation of IFN-γ. Recent studies have demonstrated that TNF-α level can predict severity of illness. Autoantibody expression in scrub typhus could be a result of molecular mimicry and a non-specific activation of the immune system. Inflammation and stimulation of polyclonal antibody formation probably induces autoantibody formation against self-antigens. The speckled pattern, which is usually seen in systemic auto-immune diseases such as lupus, mixed connective tissue disease, polymyositis and Sjogren’s syndrome was observed in the scrub typhus patients. However, the speckled pattern was also universally observed in patients with other infections.

The decrease in antibody titres during the convalescent phase supports the view that autoantibody formation is a transient phenomenon during the active phase of the infection. In a study of African immigrants, the presence of ANA was attributed to, living in an infectious environment. In this study, none of the ANA positive subjects developed any symptoms of autoimmune disease during a mean follow up of 18 months.

Thus, ANAs in scrub typhus may be an epiphemomenon. This is supported by the fact that positivity resolves with resolution of the infection. This may be akin to what happens with an acute phase reactant, in that it is a non-specific marker of an infective process. On the other hand, the association of ANA with acute respiratory distress syndrome, hepatic dysfunction and neurological manifestations, was albeit weak, suggesting that specific ANAs may play a role in the pathogenesis of organ dysfunction.

This study is the first to have assessed the correlation of disease severity and outcomes with the presence of autoantibodies in scrub typhus. The prospective nature of the study allowed for analysis of convalescent sera thus, demonstrating the transient nature of autoantibody formation. None of the patients in this study developed autoimmune disease during follow up.

Limitations

The study had few limitations meriting mention. The sample size was relatively small and this could have limited the association between ANA and organ dysfunction. Strain virulence of Orientia and genetic variation was not explored in this study and these could have been potential confounding factors. The control group was heterogeneous and it was not possible to recruit perfectly age-sex matched controls at the time of recruitment of cases. The high prevalence of ANA in this study, in cases and controls, could have been an overestimation, due to the use of a commercial kit that assessed IgG, IgM and IgA antibodies. Most laboratories perform ANA tests that detect the IgG isotype, as it is more relevant in the screening for autoimmune diseases, and IgM isotype antinuclear antibodies are seen in normal individuals and are non-specific. Convalescent samples for ANA could not be collected in 15 survivors despite multiple efforts.

This study highlights the need for further studies to understand the underlying immune mechanisms driving autoantibody formation in scrub typhus. Studies which look at humoral immunity, with analysis of immune markers along with complement levels, may throw more light on the pathogenesis of this infectious vasculitis.

CONCLUSION

The prevalence of ANA positivity was 53.9% in patients admitted to the hospital with scrub typhus infection. Its disappearance during the convalescent phase suggests that it may be a transient phenomenon. The association with organ dysfunction warrants further study of the mechanisms and impact of autoantibody formation in scrub typhus.

Conflict of interest: None to declare.

REFERENCES

1. Tamura A, Ohashi N, Urakami H, Miyamura S. Classification of Rickettsia tsutsugamushi in a new genus, Orientia gen. nov., as Orientia tsutsugamushi comb. nov. Int J Syst Bacteriol 1995; 45(3): 589–91.
2. Kelly DJ, Fuerst PA, Ching W-M, Richards AL. Scrub typhus: The geographic distribution of phenotypic and genotypic variants of Orientia tsutsugamushi. Clin Infect Dis 2009; 48(3): S203–30.
3. Chrispal A, Boorugu H, Gopinath KG, Prakash JAJ, Chandy S, Abraham OC, et al. Scrub typhus: An unrecognized threat in South India—Clinical profile and predictors of mortality. Trop Doct 2010; 40(3): 129–33.
4. Varghese GM, Trowbridge P, Janardhanan J, Thomas K, Peter JV, Mathews P, et al. Clinical profile and improving mortality trend of scrub typhus in South India. Int J Infect Dis 2014 ; 23: 39–43.
5. Rajapakse S, Rodrigo C, Fernando D. Scrub typhus: Pathophysiology, clinical manifestations and prognosis. Asian Pac J Trop Med 2012; 5(4): 261–4.
6. Tan EM, Feltkamp TE, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, et al. Range of antinuclear antibodies in “healthy” individuals. Arthritis Rheum 1997; 40(9): 1601–11.
7. Peter JV, Griffith MF, Prakash JAJ, Chrispal A, Pichamuthu K, Varghese GM. Antinuclear antibody expression in severe scrub typhus infection: Preliminary observations. J Glob Infect Dis 2014; 6(4): 195–6.
8. Ferreira FL, Bota DP, Bross A, Mélot C, Vincent JL. Serial evaluation of the SOFA score to predict outcome in critically ill
9. Knaus WA, Wagner DP, Draper EA, Zimmerman JE, Bergner M, Bastos PG, et al. The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. *Chest* 1991; 100(6): 1619–36.

10. Sengupta M, Benjamin S, Prakash JA. Scrub typhus continues to be a threat in pregnancy. *Int J Gynaecol Obstet* 2014; 127(2): 212.

11. Racoubian E, Zubaid RM, Shareef MA, Almawi WY. Prevalence of antinuclear antibodies in healthy Lebanese subjects, 2008–2015: A cross-sectional study involving 10,814 subjects. *Rheumatol Int* 2016; 36(9): 1231–6.

12. Rigon A, Sosa P, Zennaro D, Iannello G, Afeltra A. Indirect immunofluorescence in autoimmune diseases: Assessment of digital images for diagnostic purpose. *Cytometry B Clin Cytom* 2007; 72(6): 472–7.

13. Hommel B, Charuel J-L, Jaureguiberry S, Arnaud L, Courtin R, Kassab P, et al. Chronic malaria revealed by a new fluorescence pattern on the antinuclear autoantibodies test. *PloS One* 2014; 9(2): e88548.

14. Cainelli F, Betterle C, Vento S. Antinuclear antibodies are common in an infectious environment but do not predict systemic lupus erythematosus. *Ann Rheum Dis* 2004; 63(12): 1707–8.

15. Adebajo AO, Charles P, Maini RN, Hazleman BL. Autoantibodies in malaria, tuberculosis and hepatitis B in a west African population. *Clin Exp Immunol* 1993; 92(1): 73–6.

16. Lee J-H, Cho N-H, Kim S-Y, Bang S-Y, Chu H, Choi M-S, et al. Fibronectin facilitates the invasion of *Orientia tsutsugamushi* into host cells through interaction with a 56-kDa type-specific antigen. *J Infect Dis* 2008; 198(2): 250–7.

17. Mansueto P, Vitale G, Cascio A, Seidita A, Pepe I, Carroccio A, et al. New insight into immunity and immunopathology of Rickettsial diseases. *Clin Dev Immunol* 2012; 2012: 967852.

18. Kundin WD, Liu C, Harmon P, Rodina P. Pathogenesis of scrub typhus infection (*Rickettsia tsutsugamushi*) as studied by immunofluorescence. *J Immunol* 1964; 93(5): 772–81.

19. Iwasaki H, Mizoguchi J, Takada N, Tai K, Ikegaya S, Ueda T. Correlation between the concentrations of tumor necrosis factor-α and the severity of disease in patients infected with *Orientia tsutsugamushi*. *Int J Infect Dis* 2010; 14(4): e328–33.

20. Satoh M, Mercado MV-D, Chan EKL. Clinical interpretation of antinuclear antibody tests in systemic rheumatic diseases. *Mod Rheumatol* 2009; 19(3): 219–28.

21. Litwin CM, Binder SR. ANA testing in the presence of acute and chronic infections. *J Immunoassay Immunochem* 2016; 37(5): 439–52.

22. Egner W. The use of laboratory tests in the diagnosis of SLE. *J Clin Pathol* 2000; 53(6): 424–32.

23. Williams WM, Isenberg DA. A cross-sectional study of anti-DNA antibodies in the serum and IgG and IgM fraction of healthy individuals, patients with systemic lupus erythematosus and their relatives. *Lupus* 1996; 5(6): 576–86.

Correspondence to: Dr Maria Koshy, Department of Medicine, Christian Medical College, Vellore–632002, India.
E-mail: shrutikoshy@gmail.com

Received: 15 February 2017 Accepted in revised form: 14 March 2018