



ORIGINAL RESEARCH PAPER

SCRUB TYPHUS-ASSOCIATED COLD AUTOAGGLUTINATION AND HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS- A RARE HEMATOLOGICAL PRESENTATION OF AN OTHERWISE INDOLENT DISEASE: A CASE REPORT

Pathology,

KEY WORDS: Scrub Typhus, Hlh, Cold Autoimmune Hemolytic Anemia

Ishaan Thassu	Department of Pathology, Apollo Multispeciality Hospitals, Kolkata
Pritha Chatterjee	Department of Hematology, Apollo Multispeciality Hospitals, Kolkata
Anirban Kundu	Department of Hematology, Apollo Multispeciality Hospitals, Kolkata
Atoshi Basu*	Department of Hematology, Apollo Multispeciality Hospitals, Kolkata *Corresponding Author

ABSTRACT

Scrub typhus, a mite born bacterial infection caused by *Orientia tsutsugamushi*, commonly presents with flu-like symptoms, with an indolent clinical course. Life threatening complications like multi-organ failure are rare and may occur as a consequence of secondary hemophagocytic lymphohistiocytosis (HLH). Despite being an uncommon manifestation, the incidence of HLH in scrub typhus has increased in recent years and is a cause of poor clinical outcome and high mortality. In addition, hemolytic anemia in scrub typhus is also an uncommon finding, with rare cases of acute intravascular hemolysis documented in patients with Glucose-6-phosphate dehydrogenase (G6PD) deficiency. Cold agglutination in scrub typhus is rare. The underlying mechanism may be attributed to the production of IgM autoantibodies against "I" antigen of red blood cells as a result of immune triggers due to the underlying infection. We describe the case of 47-year-old female patient who presented to the emergency in a state of shock and altered sensorium. The laboratory investigations revealed autoagglutination with a positive Coombs test and HLH secondary to scrub typhus.

INTRODUCTION

Scrub typhus is caused by *Orientia tsutsugamushi*, a gram-negative bacillus of the *Rickettsiaceae* family. It is chiefly transmitted via the bites of infected trombiculid mite larvae. While scrub typhus infection commonly presents with fever, headache, myalgia, and flu-like symptoms, rarely it can lead to pneumonia, acute respiratory failure, shock, meningoencephalitis, and diffuse intravascular coagulation (DIC) [1].

Hemophagocytic lymphohistiocytosis (HLH) is a rare and dreaded complication occurring in a small percentage of cases with scrub typhus. HLH is a systemic inflammatory condition that develops as a result of cytokine overproduction and is associated with a very high mortality and inferior outcomes [2]. Clinical suspicion usually guides appropriate laboratory investigations and early detection of HLH proves to be lifesaving.

Hemolytic anemia is also an uncommon manifestation in scrub typhus, with episodic acute intravascular hemolysis being described in patients with Glucose-6-phosphate dehydrogenase (G6PD) deficiency [3]. The occurrence of cold agglutination in scrub typhus has not been described in literature to the best of our knowledge. Cold autoimmune hemolytic anemia (cAIHA) is primarily a clonal B-cell lymphoproliferative disorder, while secondarily, it may be attributed to malignancy, infection, and/or autoimmune disorders. The incidence of cAIHA in scrub typhus remains largely undocumented, with the underlying mechanism being IgM autoantibodies (against "I" antigen of red blood cells) mediated hemolysis. The immune triggers remain unknown; however may be attributed to the underlying scrub typhus infection [1,4,5].

We present the case of a 47-year-old female patient who presented to the emergency with altered sensorium and hypovolemic shock, possibly due to HLH secondary to scrub typhus. Laboratory investigations confirmed the scrub typhus infection along with secondary HLH and also revealed cold agglutination. In this case report, we describe the relevant findings with an attempt to explain the underlying pathophysiology.

Case presentation

A 47-year-old female patient with history of hypertension,

presented to the emergency with altered sensorium and persistent hypotension requiring vasopressor support.

Clinical history revealed an earlier visit to an outside hospital with complaints of fever, cough, and cold associated with chills, generalized weakness, recurrent loose stools and urinary incontinence. During her initial hospitalization, she had developed septic shock complicated by acute kidney injury (AKI) and pancytopenia, necessitating vasopressor support. She received broad-spectrum antibiotics and transfusion of two units of packed red blood cells (PRBCs) before being referred to our tertiary care center.

Laboratory investigations demonstrated pancytopenia (hemoglobin 6.2 g/dL, total leukocyte count 2,700/ μ L, platelet count 25,000/ μ L), elevated C-reactive protein (CRP)- 7.1 mg/dL, ferritin- 5,770 ng/mL, lactate dehydrogenase (LDH)- 1,099 U/L, prothrombin time (PT)- 18.0 seconds, International Normalized Ratio (INR)- 1.36, and D-dimer- 2,231 ng/mL, with hypofibrinogenemia (fibrin- 112 mg/dL), a normal activated normal thromboplastin time (APTT)- 28.3 seconds and serum triglyceride of 160 mg/dL. Overall clinical and laboratory parameters were suggestive of DIC. Peripheral blood smear examination demonstrated red blood cell agglutination, which resolved after incubation at 37°C for 2 hours [Figure 1 and Figure 2].

This was associated with spurious hematological parameters in the complete blood counts (CBC) which normalized following warming of the sample, as illustrated in Table 1. Immunohematological workup showed autoagglutination; direct antiglobulin test (DAT) performed by the conventional tube technique showed a 2+ positive reaction [Figure 3A]. Gel card method demonstrated isolated C3d positivity [Figure 3B] with a negative IgG reaction and thermal amplitude testing demonstrated strong agglutination at 4°C (4+), weak agglutination at 22°C (1+), and no agglutination at 37°C; consistent with cold agglutinin-mediated autoimmune hemolysis [Figure 4A, 4B & 4C]. **Indirect Coombs Test (IAT)** showed **mixed-field agglutination** [Figure 5A & 5B]. Aggressive supportive management was instituted in view of coagulopathy and included intravenous fluid resuscitation, two units of PRBC transfusion, six units random donor platelets (RDPs), ten units of cryoprecipitate, and two grams of fibrinogen concentrate for persistent hypofibrinogenemia secondary to DIC.

Table 1: Pre- and Post-warming Red Blood Cell (RBC) Indices

Parameter	Pre-warming	Post-warming
RBC Count	1.9 million/ μ L	3.2 million/ μ L
MCV (Mean Corpuscular Volume)	136 fL	94 fL
MCH (Mean Corpuscular Hemoglobin)	28 pg	30 pg
MCHC (Mean Corpuscular Hemoglobin Concentration)	38 g/dL	32 g/dL

Bone marrow examination was performed in view of pancytopenia, and revealed hemophagocytosis [Figure 6A,B, C,D,E & F]. Infectious disease screening showed scrub typhus serology positivity, while leptospira, chikungunya, and -D-glucan assay were negative. Empirical antimicrobial therapy was escalated to include intravenous meropenem while doxycycline and azithromycin were added against *Orientia tsutsugamushi*. Given the constellation of clinical and laboratory findings, the patient was evaluated for HLH and as the patient fulfilled six of the eight HLH-2004 diagnostic criteria (namely splenomegaly, fever, cytopenia, hypofibrinogenemia, hemophagocytosis, hyperferritinemia), a diagnosis of HLH was established and intravenous dexamethasone therapy initiated [2].

Although she showed brief improvement with discontinuation of vasopressors and gradual weaning of respiratory support, her clinical condition subsequently worsened with recurrent shock and progressive multiorgan dysfunction syndrome (MODS). She developed sudden hypotension followed by asystolic cardiac arrest. Cardiopulmonary resuscitation (CPR) was performed according to Advanced Cardiovascular Life Support (ACLS) guidelines; however, return of spontaneous circulation could not be achieved despite three cycles of high-quality CPR, and the patient was declared deceased.

DISCUSSION

Scrub typhus, caused by *Orientia tsutsugamushi* and transmitted by chiggers (larval trombiculid mites), remains an important cause of acute febrile illness across the Asia-Pacific region. Frequently, an eschar is identified at the site of chigger feeding and helps in early recognition of the disease [1,6]. Failure to identify the disease may however be associated with worsening of clinical outcomes, including development of vasculitis, HLH, DIC and MODS [7-9]. Our case presented with hypovolemic shock and altered sensorium and an extensive infectious disease screening led to the identification of underlying scrub typhus. The patient showed a stormy clinical course, in contrast to the usual self-limiting course described in literature [2]. In addition, the patient showed features of secondary HLH and cAIHA, both of which were associated with the rapid deterioration and poor treatment outcomes in the index case.

Secondary HLH is a rare, yet dreaded complication of scrub typhus infection and is diagnosed based on both laboratory and clinical criteria. The underlying pathophysiology may be attributed to uncontrolled macrophage activation, secondary to *Orientia tsutsugamushi* infection. Sustained cytokine release and secondary cytotoxic T-cell inactivation usually follow. Worthwhile to note is the difference in the levels of cytokines released, while comparing adult and pediatric populations. Pediatric patients have been shown to release a significantly lower level of interferons (IFN) and interleukins (IL), namely IL-2, IFN- γ , IL-4, and IL-6. The differences in cytokine release are often assuming with a differential susceptibility in adults and children [2, 10]. Our case fulfilled 6 of the 8 HLH-2004 diagnostic criteria, including fever ($>38.5^{\circ}\text{C}$ for 12 days), splenomegaly, cytopenias affecting all three peripheral blood cell lineages, hypofibrinogenemia

(0.4 g/L), hyperferritinemia (5,770 ng/mL), and hemophagocytosis in the bone marrow, thereby confirming the diagnosis of HLH. The patient was managed with intravenous dexamethasone, meropenem along with doxycycline and azithromycin. Despite the early intervention, the patient eventually succumbed to disease complications. Intravascular hemolysis in patients with G6-PD deficiency has been described in scrub typhus, however cAIHA remains a rare presentation. The exact incidence cAIHA in scrub typhus patients has not been described in medical literature to the best of knowledge. While the exact cause remains unknown, formation of IgM antibodies against the "I" antigen due to the underlying infection may be the probable mechanism. These IgM antibodies at low temperatures fix complement onto the RBC surface; as the cells re-enter the warm central circulation, the IgM dissociates but C3d remains bound—resulting in DCT positivity for complement [3-5]. Our case showed DAT positivity (2+) by the conventional tube technique. Further evaluation by monospecific gel card testing revealed isolated C3d positivity with a negative IgG reaction, along with strong agglutination at 4°C (4+) on thermal amplitude testing, thereby indicating complement deposition on the red blood cells and confirming a cold agglutinin mediated disease. The features and probable underlying mechanism have been demonstrated in a flowchart [Figure 7].

CONCLUSION

Scrub typhus usually presents with an indolent clinical course; however delay in diagnosis may be accompanied by life threatening complications such as HLH, or more rarely haemolytic anemia. Persistent immune activation and cytokine dysregulation likely contribute to secondary HLH and complement-mediated hemolysis. This case highlights that a high index of suspicion for HLH and cAIHA is essential in patients with severe scrub typhus presenting with persistent fever, cytopenias, coagulopathy, and hemolysis, as early recognition and timely initiation of appropriate antimicrobial, immunomodulatory, and supportive therapy may improve outcomes.

Author information

Names of authors-Ishaan Thassu, Pritha Chatterjee, Anirban Kundu, Atoshi Basu

Affiliations-

1. Junior Resident (Academic), Department of Pathology, MBBS, Apollo Multi-speciality Hospitals, Kolkata
2. Junior Resident (Academic), Department of Pathology, MBBS, Apollo Multi-speciality Hospitals, Kolkata
3. Associate Consultant, Department of Hematology, MBBS, MD (Pathology), PDF (Laboratory Hematology), Apollo Multi-speciality Hospitals, Kolkata
4. Senior Consultant and Laboratory Director, Department of Hematology, MBBS, MD (Pathology), Apollo Multi-speciality Hospitals, Kolkata

Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Ishaan thassu, Pritha Chatterjee , Anirban Kundu, Atoshi Basu Acquisition, analysis, or interpretation of data: Anirban Kundu, Atoshi Basu Drafting of the manuscript: Ishaan thassu, Pritha Chatterjee , Anirban Kundu Critical review of the manuscript for important intellectual content: Ishaan thassu, Pritha Chatterjee , Anirban Kundu, Atoshi Basu

Supervision: Anirban Kundu, Atoshi Basu

Corresponding author

Correspondence to Pritha Chatterjee
Email- prithachatterjee0907@gmail.com

Ethics declarations

Funding

The authors did not receive support from any organization for the submitted work.

Conflict of interest

The authors declare no competing interests.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors beyond routine diagnostic procedures.

Informed consent

Informed consent was waived due to the retrospective and de-identified nature of the report, in accordance with institutional policy.

Consent for publication

Consent for publication was waived because all identifying information was removed and the case was fully anonymized.

Figure and legends

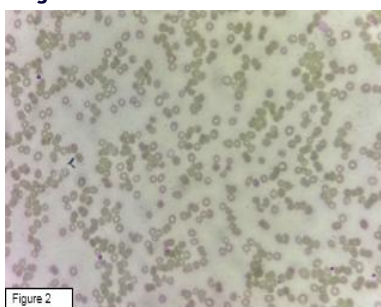


Figure 1. Peripheral blood smear examined at ($\times 400$) magnification showing marked red blood cell agglutination pre warming.

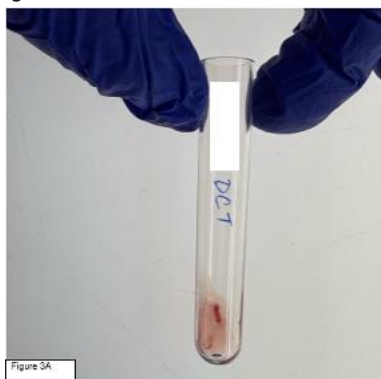


Figure 2. Peripheral blood smear ($\times 400$) demonstrating resolution of red blood cell agglutination following incubation at 37°C .

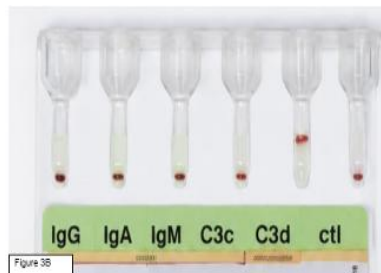


Figure 3A. Direct antiglobulin test (DAT) performed by the conventional tube technique demonstrating a **2+ positive reaction**.

Figure 3B. Monospecific direct antiglobulin test (DAT) by gel card method demonstrating **isolated C3d positivity with negative IgG, IgA, IgM, and C3c reactions**.

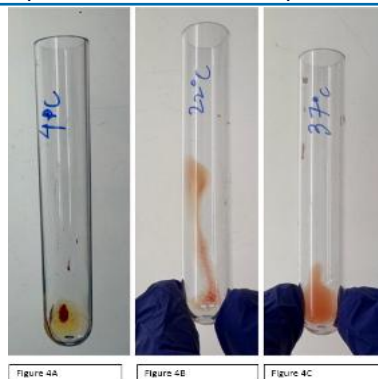


Figure 4A-C. Thermal amplitude testing demonstrating **strong agglutination at 4°C (4+) (A), weak agglutination at 22°C (1+) (B), and no agglutination at 37°C (C).**

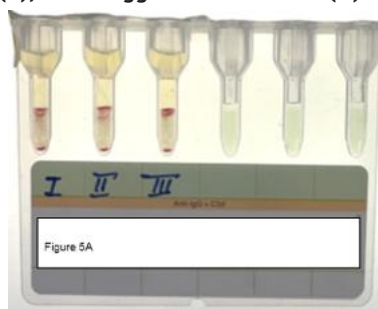


Figure 5A-B. Indirect antiglobulin test (IAT) performed by the conventional tube technique demonstrating **mixed-field agglutination**.

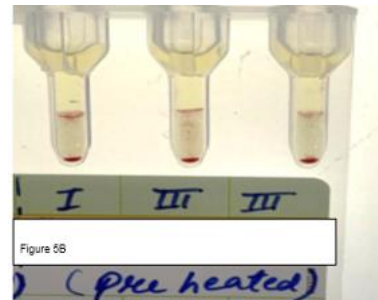
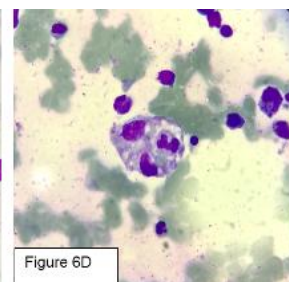
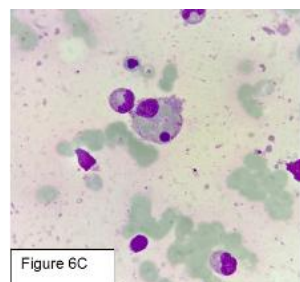
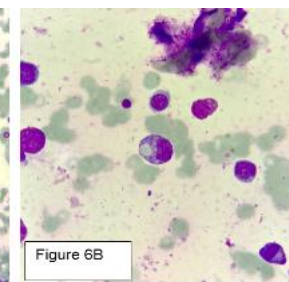
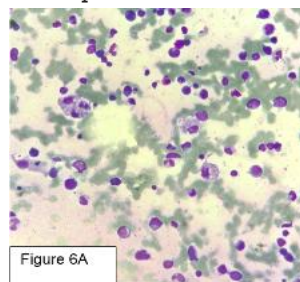


Figure 6A-F. Bone marrow aspirate demonstrating **hemophagocytosis**. **(A)** $400\times$ showing hemophagocytic activity. **(B-F)** $1000\times$, oil immersion, demonstrating histiocytes exhibiting active hemophagocytosis of hematopoietic cells.



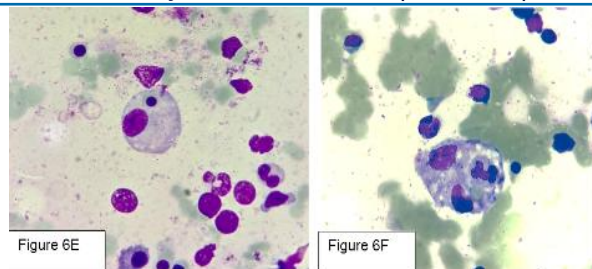


Figure 7. The key features and the probable underlying mechanism are illustrated in the accompanying flowchart .

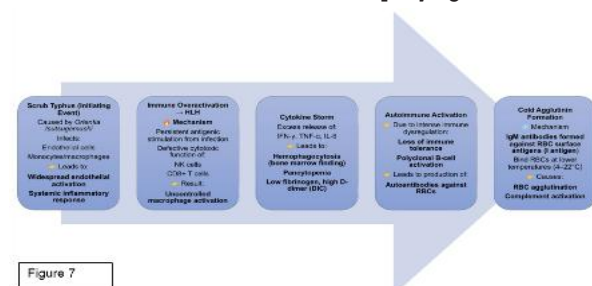


Figure 7

REFERENCES

- Peter JV, Sudarsan TI, Prakash JA, Varghese GM. Severe scrub typhus infection: Clinical features, diagnostic challenges and management. *World J Crit Care Med.* 2015 Aug 4;4(3):244-50. doi:10.5492/wjccm.v4.i3.244.
- Naot T, Morita M, Kawakami T, Fujimoto S. Hemophagocytic Lymphohistiocytosis Associated with Scrub Typhus: Systematic Review and Comparison between Pediatric and Adult Cases. *Trop Med Infect Dis.* 2018 Feb 14;3(1):19. doi:10.3390/tropicalmed3010019.
- Shukla R, Shrestha M, Bhattarai CD, Lamichhane K, Yadav P, Tamatta D. Scrub Typhus Infection Precipitating Hemolysis in a Patient With G6PD Deficiency: A Case Report. *Clin Case Rep.* 2025 Jan 24;13(2):e70142. doi:10.1002/ccr3.70142.
- Despotovic JM, Kim TO. Cold AIHA and the best treatment strategies. *Hematology Am Soc Hematol Educ Program.* 2022 Dec 9;2022(1):90-95. doi:10.1182/hematology.2022000369.
- Ganguly D, Chandra A, Maitra S, Malakar SL. Acute intravascular haemolysis associated with scrub typhus. *BMJ Case Rep.* 2024 Jul 2;17(7):e261003. doi:10.1136/bcr-2024-261003.
- Jeong YJ, Kim S, Wook YD, Lee JW, Kim KI, Lee SH. Scrub typhus: clinical, pathologic, and imaging findings. *Radiographics.* 2007;27:161-172. doi:10.1148/rg.271065074.
- Kim S.J., Chung I.K., Chung I.S., Song D.H., Park S.H., Kim H.S., Lee M.H. The clinical significance of upper gastrointestinal endoscopy in gastrointestinal vasculitis related to scrub typhus. *Endoscopy.* 2000;32:950-955. doi:10.1055/s-2000-9621.
- Seong S.Y., Choi M.S., Kim I.S. Orientia tsutsugamushi infection: Overview and immune responses. *Microbes Infect.* 2001;3:11-21. doi:10.1016/S1286-4579(00)01352-6.
- Strickman D., Smith C.D., Corcoran K.D., Ngampochjana M., Watcharapichat P., Phulsuksombati D., Tanskul P., Dasch G.A., Kelly D.J. Pathology of Rickettsia tsutsugamushi infection in Bandicota savilei, a natural host in Thailand. *Am J Trop Med Hyg.* 1994;51:416-423. doi:10.4269/ajtmh.1994.51.416.
- Lilic D, Cant AJ, Abinun M, Calvert JE, Spickett GP. Cytokine production differs in children and adults. *Pediatr Res.* 1997 Aug;42(2):237-40. doi:10.1203/00006450-199708000-00018.