INTRODUCTION

Pseudoneutropenia or low leucocyte count secondary to leucoagglutination is caused by ethylene diamine tetra acetic acid (EDTA) or cold agglutinins and is seen in benign and malignant disorders. We report a 34-year-old lady who was admitted with fever, vomiting, respiratory distress and productive cough. Complete blood count (CBC) at initial presentation revealed low haemoglobin (11.6 g/dL), total leucocyte count (TLC) (5900/mm$^3$) with 50% polymorphs. Peripheral blood smear showed leucocytes in clusters. Another sample was asked for in citrate anticoagulant which showed a TLC of 5900/mm$^3$ with 50% polymorphs and evidence of auto agglutination. Another collected in a pre-warmed ethylene diamine tetra acetic acid (EDTA) tube, CBC showed a TLC of 9800/mm$^3$ with 39% neutrophils suggestive of pseudoneutropenia due to cold agglutinins.

CASE REPORT

A 34-year-old lady presented with complaints of throat pain associated with fever, vomiting, respiratory distress and productive cough. There was no family history of similar complaints. Her past history and personal history were unremarkable. On general physical examination, the patient appeared ill; blood pressure, pulse and respirations were within normal limits. She was febrile (oral temperature 100.2 °F). Bilateral crepitations were heard on chest auscultation. Other findings on physical examination were unremarkable.

On admission her biochemical and haematological investigations were normal except for complete blood count (CBC) vide infra. Chest radiograph was normal. High resolution computed tomography (HRCT) showed multiple small nodular lesions in both lungs, with a ‘tree-in-bud’ appearance suggestive of infective pathology. Sputum smear did not reveal any organisms on Gram’s staining and sputum for bacterial culture was sterile. Bronchial washings cytology did not reveal malignant cells. Bronchial washings did
not reveal acid-fast bacilli (AFB); culture and sensitivity was sterile. The patient tested negative for human immunodeficiency virus (HIV), hepatitis, typhoid, rickettsia and brucella infections. On serological testing, peripheral smear did not reveal malarial parasite.

CBC was performed by using three-part automated haematology analyzer on sample collected in EDTA on the same day. It revealed a low haemoglobin (11.6 g/dL), total leucocyte count (TLC) of 5900/mm$^3$, platelet count of 51,500/mm$^3$, with neutrophils of 50% and lymphocytes of 5.1x10$^9$/L. Peripheral smear made from an EDTA anticoagulated blood sample specimen revealed leucocytes in clusters and neutrophil aggregates (Figure 1). A sample in citrate anticoagulant, was asked for to rule out EDTA mediated neutrophil aggregates. This sample showed a WBC count of 59,000/mm$^3$ with neutrophils 50% and evidence of autoagglutination. At this time a sample was collected in pre-warmed (37 °C) EDTA tube. This sample showed TLC of 9800/mm$^3$ with neutrophils 39%; there was no evidence of autoagglutination and peripheral smear showed dispersed neutrophils and no leucoagglutination (Figure 2). The presence of autoagglutination and leucoagglutination at room temperature and disappearance at 37 °C shows the presence of cold agglutinins. Based on the patient clinical condition and initial lab reports we tested the patient sample for antibodies against *Mycoplasma pneumoniae* antigen, which was strongly positive.

The patient was diagnosed to have pseudoneutropenia due to cold agglutinin leucoagglutination caused by *Mycoplasma pneumoniae* infection.

**DISCUSSION**

Cold agglutinin associated leucoagglutination in peripheral blood is not uncommon. Rarely seen in normal individuals, it is more commonly found in the clinical setting of infections, sepsis, lymphoproliferative disorders, alcoholic liver disease and autoimmune diseases. This finding gives clue for an underlying clinical condition and helps in diagnosis and management.

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**Figure 1:** Photomicrograph of the peripheral smear showing leucoagglutination, aggregated neutrophils (Leishman, × 40)

**Figure 2:** Photomicrograph of the peripheral smear after collection of sample in pre-warmed tube (Leishman, × 40)
There are three types of antierythrocyte autoantibodies which exhibit distinctive serologic properties and result in characteristic clinical disorders. IgG warm antibodies attach to erythrocytes at 37 °C, immunoglobulin M (IgM) cold autoantibodies clump red blood cells (RBCs) at lower temperatures and immunoglobulin G (IgG) Donath-Landsteiner antibodies bind to RBC membranes in cold and causes haemolysis at 37 °C.4

In the present case cold agglutinins are formed due to *Mycoplasma pneumoniae* infection. In the cold autoantibody type occurring with *Mycoplasma pneumoniae* cross-antigenicity between mycoplasmal cell wall and I antigen present on red cell membrane has been suggested.5 In this case cold agglutinins are specific to I antigen. The I antigen is present on RBCs, leucocytes, lens, stomach etc.6 The IgM cold agglutinins attached to I antigen on red cells and leucocytes causing autoagglutination of red cells and leucoagglutination which leads to pseudoneutropenia.

Leucoagglutination is associated with either a spurious leucopenia or an underestimation of hyperleucocytosis. This can adversely affect management decisions in terms of unnecessary treatment and management of leucopenia or ignoring a very high leucocyte count that may be an indicator for underlying serious disease.7

REFERENCES