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A Normal Absolute Neutrophils Count in Bacterial Infection: A Precaution Step Needed

Abstract—The rare condition of neutrophils agglutination in-vitro may give inaccurate neutrophils count. We reported a case of a 71-year-old female admitted with a diagnosis of extended-spectrum beta-lactamase (ESBL) *Escherichia Coli* (E.coli) septicaemia secondary to the left lower limb cellulitis. Despite underlying infection, total white blood cell count (TWBC) and absolute neutrophil count remained in a normal range. An accidental finding of full blood picture (FBP) using K2 ethylenediaminetetraacetic acid (EDTA) tube presented numerous neutrophils agglutination. Further tests were carried out using citrated and heparinised tubes and tested at room temperature, 4 °C, and 37 °C. The findings showed a significant reduction in neutrophils agglutination in both citrated and heparinised tubes, including when tested at room temperature (RT) and 4 °C. Surprisingly, at 37 °C, no neutrophil agglutination was observed. This indicates that temperature-dependent anti-neutrophil IgM antibody may be found in the patient's serum that reacted with the EDTA anticoagulant. In conclusion, white blood cells (WBC) agglutination has been associated with malignancy, sepsis, liver disease, and autoimmune disease. Thus, a baseline FBP is recommended, especially in these categories of patients to optimise their clinical management.

Keywords— neutrophil count, neutrophil agglutination, full blood picture.

1 INTRODUCTION

White blood cells (WBC) agglutination in-vitro is a rare condition. It may lead to pseudoleukopenia or inaccurate total white cell count in an expected leukocytosis patient. Thus, this may affect the clinical interpretation of the laboratory result. Most reports of WBC agglutinations are based on a case report basis [1,2,3]. Here is another rare case of WBC agglutination that has been identified, which specifically involved neutrophils, accidentally detected by full blood picture (FBP). The following provides the details of the case.

2 CASE REPORT

A 71-year-old Chinese female was admitted to the medical ward on 31st December 2016 for intermittent fever with chill and rigour for two weeks before presentation. The symptoms were associated with bilateral lower limb weakness and swelling. She was bed-bound for the past three weeks before admission. There was no history of trauma or fall. She has chronic illnesses, namely diabetes mellitus, chronic kidney disease (CKD), and right knee osteoarthritis but defaulted follow up. She had a history of hysterectomy in a private hospital for cervical carcinoma in 2013. Currently, she is not on any regular medication. She is single, nulliparous, unemployed, and stays with a sister.

On examination, she was alert, orientated, and pink at room air. Vital signs were stable. Bilateral lower limbs were swelling associated with erythematous skin and superficial ulcer on her left shin. Neurological examination revealed reduced muscle tone and power of bilateral lower limbs. Other systemic examinations were unremarkable.

An initial full blood count showed anaemia (Hb8.4g/dl) with a normal total WBC of $7.8 \times 10^3/\mu\text{L}$ (the absolute neutrophil count was $6.4 \times 10^3/\mu\text{L}$). Renal function test showed deranged results with hypokalaemia (2.9 mmol/L), uraemia (17.1 mmol/L), and high creatinine level (193 mmol/L) which correspond to her CKD. Blood culture and sensitivity were revealed to be positive for extended-spectrum beta-lactamase (ESBL) *Escherichia Coli* with antibiotic sensitivity toward amikacin, Imipenem, meropenem, gentamicin, and ertapenem. Otherwise, other investigations were normal.

Serum ferritin, serum iron, and FBP were ordered as part of the investigation of anaemia on the day of admission. Serum ferritin was high (787 ug/L), but serum iron was low (2.9 umol/L). A blood sample was collected using a K2 ethylenediaminetetraacetic acid (EDTA) tube, and Wright's stain was used for slide preparation. The result showed the presence of significant neutrophils agglutination with other

morphological features suggestive of infection (Figure 1).

Further tests were carried out to investigate the cause of neutrophil agglutinations. FBP was repeated on Day 7 of admission using a citrated tube (Figure 2) and heparinised tube. FBP was also repeated using a blood sample which was

incubated at room temperature (RT), 4°C, and 37°C (Figure 3) for 5 minutes. Findings showed significantly reduced neutrophil agglutinations in both citrated and heparinised tubes, including when tested at RT and 4°C. Surprisingly, neutrophil agglutination was disappeared when using a blood sample incubated at 37°C.

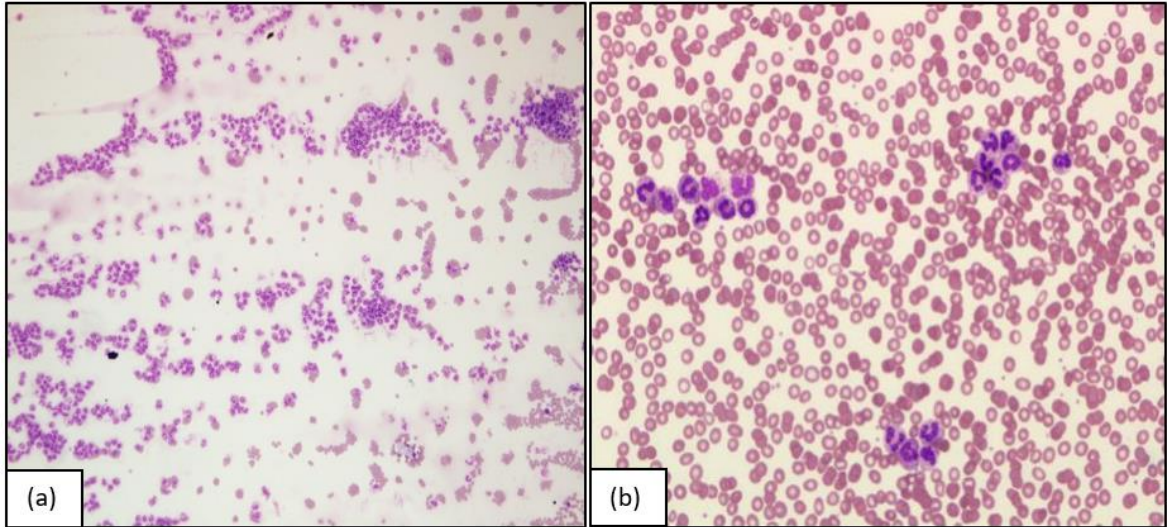


Figure 1: Initial FBP showed numerous neutrophils agglutination in EDTA tube (200x magnification for (a) at the tail area and 400x magnification (b) at the body area for smear

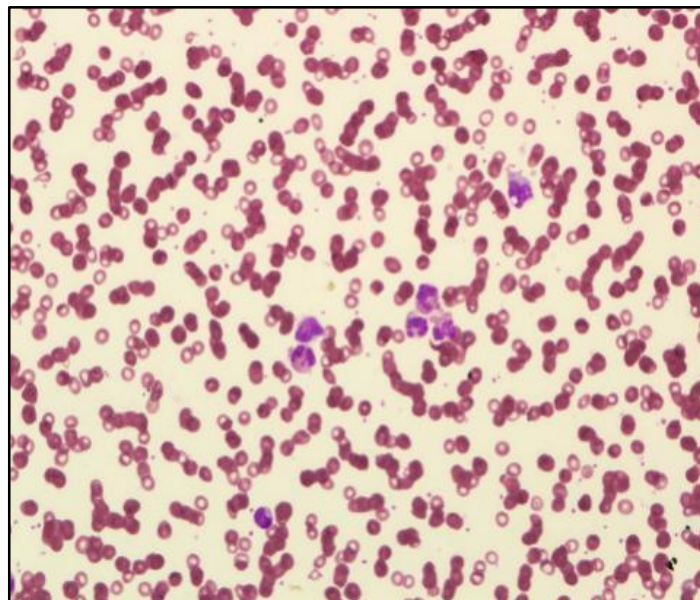


Figure 2: Mild neutrophil agglutination using a citrated tube (400x magnification) at the body area of the smear

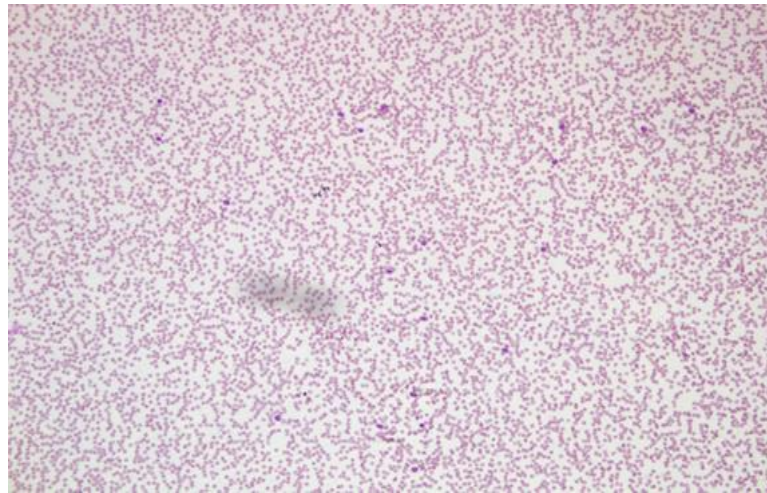


Figure 3: Absence of neutrophil agglutination at 37°C (200x magnification) at the body area of the smear

The patient's total WBC and neutrophils count remain normal although neutrophilia was expected in bacterial infection. Repeated WBC count and neutrophil count from a sample using EDTA, sodium citrate, and heparinised tube were within a normal range. Manipulation of temperature also showed similar findings (Table 1).

Serum immunoglobulin subclass IgM and IgG were 1.33 g/l (0.45-1.45) and 14.8 g/dl (5.5-15.0) respectively. Direct and indirect antihuman globulin test (AHG) was negative. The patient was observed in the ward. Only one-episode spiking of

temperature (38.5°C) during admission was noted. Otherwise, vital signs were stable throughout the admission. She was started with intravenous (IV) Unasyn 750mg TDS on admission, but later at Day 5 of admission, it was changed to IV Imipenem 500mg TDS as guided by blood culture and sensitivity result. The mist potassium chloride was given because of hypokalemia and haematinic for the iron deficiency anaemia. She was discharged on 18th January 2017 after completed IV antibiotic for two weeks and clinically recovered.

Table 1: WBC count and neutrophil count using different tube and at different temperature.

*These tests were done at a room temperature

**These tests were done using EDTA tube at three different temperatures

Parameter	*EDTA	*Sodium Citrate	*Heparinised	**Room temperature	**4°C	**37°C	Normal Range
WBC count ($\times 10^3/\mu$ L)	7.7	6.9	5.8	6.3	6.1	6.0	4.1-11.4
Neutrophil ($\times 10^3/\mu$ L)	6.1	5.5	4.2	4.3	4.5	4.4	3.9-7.1

4 DISCUSSION

WBC agglutination has been associated with malignancy, sepsis, liver disease and autoimmune disease [2]. Accurate WBC count is important for monitoring response toward antibiotic in a septicemic patient. WBC count also was part of risk stratification criteria and monitoring response toward chemotherapy in haematological malignancy. In a certain group of patients such as neonates, increment in WBC may be the only abnormality detected with minimal symptom presentation. In our case, the patient was diagnosed with ESBL *E.coli* septicemia, evidenced by blood culture. The neutrophils agglutinations might have gone undetected if FBP was not sent. During admission, the patient's total WBC and neutrophils count remain normal although neutrophilia was expected in bacterial infection (Table 1). There are three possible explanations; one would be due to the presence of neutrophils agglutination itself where the machine cannot give an accurate count. The other might be due to low immune response since the patient is in her advanced age, and the third cause could be due to the complete response to the initial antibiotic given, which is the Unasyn.

The importance of knowing the true absolute neutrophils count in a patient with infection is in monitoring response to the antibiotic given. If the antibiotic is sensitive, we expect that the initial total WBC (expected to increase in infection) will slowly reduce. In this patient, the presence of neutrophils agglutination might give a false normal value of its absolute count. Here, from a medical point of view, the treating doctor might think the antibiotic is sensitive enough as evidence by the normal neutrophils count. However, the true story is that patient having resistant towards antibiotic Unasyn as evidence by blood culture as it was changed to a more sensitive antibiotic, namely Imipenem. In our case, we had proved that it was induced by EDTA tube however repeat FBP with sodium citrate and heparinised bottles did not show increased of absolute neutrophils perhaps partly due to the partial response to the initial antibiotic Unasyn given earlier.

In the previous literature review, WBC agglutination mechanism was postulated due to the use of K2-EDTA anticoagulant or temperature-dependent [1,2,4,5]. This is reflected in our case where significant neutrophils agglutination that was seen in the EDTA tube has reduced its intensity when used in citrated and heparinised tubes. The nature of its temperature-dependent

was well observed where neutrophils agglutination that was initially seen at room temperature and 4 °C had resolved entirely at 37 °C (using EDTA tube). This indicates that temperature-dependent anti-neutrophil IgM antibody (which is known to have optimum activity at cold temperature) may present in the patient's serum that reacts with EDTA anticoagulant. However, the patient's serum immunoglobulin IgM was normal, although it was on an upper border normal range value. This is probably because the immunoglobulin has reduced to a normal range as the body responded to the antibiotic given to the patient earlier. We also have excluded the presence of other atypical antibodies that might be causing similar findings. This is proven by negative direct and indirect AHG test.

With regards to her anaemia, red blood cell morphologically was normochromic (MCH 30 pg) normocytic (MCV 86 fL) with normal MCHC of 32.2 g/dL. However, the patient's iron was found reduced, which was suggestive of iron deficiency despite high serum ferritin due to its nature as an acute-phase protein. She was treated empirically with haematinic.

WBC agglutination is usually a transient phenomenon. It disappears when the underlying cause resolved. However, few cases reported persistent WBC agglutination up to 5 years duration [5]. Further plan to monitor a full blood picture in this patient was not done as she defaulted follow-up.

5 CONCLUSION

In conclusion, when the total WBC and absolute neutrophil count does not tally with the patient's clinical picture, differential count based on a blood smear is the best method to ascertain the true count. Therefore, clinicians need to be aware of the existence of this phenomenon, especially in a group of patients diagnosed with haematological malignancy, septicemic patient or any group of a patient that depends on the accurate WBC in guiding the clinical management.

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CONSENT FOR PUBLICATION

For the publication of this case report and the accompanying images, written permission was given by the patient. Institutional Review Board approval for the presentation of a single case report is not needed at the author's institution.

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