Cold-Induced Pseudoneutropenia in Human Immunodeficiency Virus Infection: First Case Report and Review of Related Articles

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Abstract Cold agglutination of erythrocyte or platelet aggregation in vitro due to cold agglutination are well recognized and extensively studied. Aggregation of leucocyte is a rare hematological phenomenon resulting in a spurious low leucocyte count performed using automated hematology analyzers. Aggregation of leucocyte may relate to malignancy, lymphoproliferative disorders, infection, liver diseases, or autoimmune disorders. It is believed that the mechanism of leucocyte aggregation is mainly related to the use of ethylene diamine tetraacetic acid (EDTA) anticoagulant or is temperature-mediated. Leucoagglutination is associated with either a spurious leucopenia or an underestimation of leucocytosis. This can adversely affect management decisions in terms of unnecessary management of leucopenia or ignoring a leucocytosis that may be indicator of an underlying serious disease. To our knowledge, we report here for the first time the occurrence of pseudoneutropenia due to temperature-mediated, EDTA-independent neutrophil agglutination in adult with human immunodeficiency virus infection.

Keywords Pseudoneutropenia · Neutrophil agglutination · EDTA · HIV · Cold agglutination

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Introduction

Automated hematology analyzers provide quick, accurate and reproducible blood cell counts. However, depending on detection methods, spurious count may occur. Spuriously low leucocyte count (pseudoleucopenia) is a rare hematological phenomenon, mainly related to agglutination of leucocytes in vitro ethylene diamine tetraacetic acid (EDTA) dependent and/or temperature (cold) mediated [1, 2]. Aggregation of neutrophils in peripheral blood smear (PSB) also referred to as leucoagglutination, neutrophils agglutination, granulocytes aggregation or neutrophil clumping, is a very rare, mostly self-limiting hematological phenomenon [3]. Leucoagglutination may relate to malignancy, lymphoproliferative disorders, infection, liver diseases, or autoimmune disorders. It is believed that the mechanism of leucocyte aggregation is mainly related to the use of EDTA anticoagulant but it is also temperature-mediated. True neutropenia in human immunodeficiency virus (HIV) infection is well documented while pseudoneutropenia has not been reported in HIV infection so far as per our knowledge. To our knowledge, we report here for the first time the occurrence of pseudoneutropenia due to temperature (cold) mediated, EDTA-independent neutrophil agglutination in adult with HIV infection.

Case Report

A 28-year-old male patient visited a hospital on December, 2013 because of mild grade fever since two and half months and cough since 10 days. There was no significant past medical, surgical or family history. There was no history of diabetes, hyperlipidemia, hypertension, and other metabolic and endocrine disorders. A general physical

Table 1 Complete Blood Cell count result

Complete blood count	Sample in EDTA as anticoagulant		Sample in sodium citrate as anticoagulant ^a	
	At room temperature (22 °C)	At 37 °C after 30 min	At room temperature (22 °C)	At 37 °C after 30 min
WBC ($\times 10^9/L$)	2.3	4.6	2.6	4.3
RBC ($\times 10^{12}/L$)	3.29	3.42	3.32	3.35
HB (g/L)	90	91	89	90
HCT (%)	29.1	30.1	29.1	29.4
MCV (fL)	88.4	88	87.7	87.8
MCH (pg)	27.4	26.6	26.8	26.9
MCHC (g/L)	309	302	306	306
PLT ($\times 10^9/L$)	182	165	188	199
RDW (%)	16.6	16.6	16.9	16.6

^a Value obtain on citrated blood sample were multiple by dilution factor to get comparable result

Fig. 1 PBS from blood sample drawn in EDTA shows an aggregate of 18–21 neutrophils in rosette like pattern ($\times 100$, oil immersion, Giemsa stain)

examination did not detect any abnormalities except the pallor.

Routine hematological investigations done on EDTA-anticoagulated blood sample on an automated hematology analyzer Sysmex KX-21 (Sysmex Corporation, Japan) revealed the WBC count, $2.3 \times 10^9/L$ and flag T2 on WBC Histogram (Table 1). However, when the PBS prepared at room temperature was examined, there was numerous neutrophil aggregates comprising of 10–20 cells (Fig. 1), yet lymphocytes showed no aggregation. When the same EDTA sample was incubated at 37 °C for 30 min, the WBC count increased to $4.6 \times 10^9/L$ (Table 1). When the PBS prepared after incubation at 37 °C was examined, all neutrophils were dispersed and no aggregation was found.

A repeat blood sample in sodium citrate as anticoagulant was evaluated, a WBC count recorded $2.6 \times 10^9/L$ at room temperature while $4.3 \times 10^9/L$ after incubation at 37 °C for 30 min (Table 1). Neutrophil aggregation was also observed in PBS when sodium citrate was used as an anticoagulant at room temperature. The PBS prepared immediately after finger prick, showed normal WBC count without neutrophil aggregation. Significant neutrophil aggregations also were observed in PBS when EDTA-anticoagulant blood from healthy donor was mixed with serum from the patient at room temperature while same mixed sample showed no aggregation at 37 °C.

Erythrocyte sedimentation rate (ESR) was 80 mm/1 h and HIV was positive for type 1. The immunoglobulin M (IgM) had increased to 3.83 g/L (normal range 0.4–2.3 g/L). The routine biochemical tests were within normal limits. Peripheral smear for malaria parasite and widal test was negative. Other laboratory investigations showed that rheumatoid factor, antistreptolysin O was within normal range. X-ray chest PA view showed apical pleural thickening and blunt costophrenic angle of left side.

Discussion

Automated hematology analyzers provide quick and accurate results in most situations. However, spurious results may be observed in several instances. Spuriously low blood cell counts may be observed because of agglutination of blood cells or cell aggregation. Cell aggregation can be classified as neutrophil aggregation, lymphocyte aggregation, leucocyte aggregation, platelet aggregation, erythrocytes aggregation, and leucocyte-platelet aggregation. Leucocyte aggregates may comprise all major WBC classes or be limited to only on class, particularly neutrophil. Aggregation of lymphocytes (Lymphoagglutination) is an extremely rare, EDTA-dependent phenomenon and is far less frequent than Neutrophils agglutination [4, 5]. Sometime, leucoagglutination is accompanied with platelet aggregation [6] or erythrocyte aggregation [7]. The phenomenon described here is distinct from artificial leucocyte aggregation (artifact) at the edge of a blood smear sometimes observed during blood smear preparation.

Neutrophil agglutination predominantly seen in adults (median age 60 years, range is 24–97 years) and very rare in children [3]. Neutrophils agglutination phenomenon may relate to different type of malignancies (e.g. Colon cancer [8], liver cell carcinoma [9] etc.), infection or inflammation (chest infection or pneumonia [3, 7, 10–12], gastroenteritis, mycoplasma, EV virus, Herpes simplex [3, 7], etc.), hepatic disorders, shock, or autoimmune disease [6]. This phenomenon is also seen in healthy persons [10]. Pure

lymphocyte agglutination has been found in lymphoproliferative disease and infections.

The infection by HIV is commonly associated with true neutropenia (absolute neutrophil count $<1.5 \times 10^9/L$). In patients infected with HIV, neutropenia is seen in the 8–50 % of them, and also have abnormalities in the neutrophil function. True neutropenia in HIV infection is well documented while pseudoneutropenia has not been reported in HIV infection so far as per our knowledge.

Although the exact reason for neutrophil agglutination in vitro has not been clarified, its relation to the use of EDTA as an anticoagulant has been described in almost all reports [3, 10, 11]. Only few cases of pseudoneutropenia due to temperature-mediated, and EDTA-independent agglutination of neutrophil has been reported as in our case [2, 8, 12]. For few cases, the artifact occurred regardless of the type of anticoagulant as in our case and warming at 37 °C did not always increase the leucocyte [1]. In our case, neutrophil agglutination was found in the blood smears when EDTA as well as sodium citrate was used as an anticoagulant. Some authors suggested that IgM antibody may play an important role in the formation of neutrophil agglutination [10, 12]. So, we can classify the neutrophil agglutination into two groups: (1) EDTA-dependent neutrophil agglutination, and (2) EDTA-independent, cold-induced neutrophil agglutination.

The formation of neutrophil agglutination is a time-dependent process, which starts gradually almost immediately after venipuncture and stabilizes after 60–90 min. Neutrophil agglutination is usually a transient phenomenon varying from days to several months, but duration of more than 5 years has been reported occasionally. In the most of reported cases of neutrophil aggregation, agglutination was confined to mature neutrophils, but rosetting of mature forms around immature neutrophils was found as well as aggregation of neutrophils with platelets. Leucocytes agglutination was induced when blood from healthy donor was mixed with serum from the patient at room temperature (22–25 °C). These results suggest that the phenomenon of in vitro leucocyte agglutination and consequent pseudoleucopenia is due to leucocyte agglutinin in serum from the patient. Some authors have reported that the use of kanamycin, polyclonal anti-M antibody, or citrate-pyridoxal 5'-phosphate-tris (CPT) anticoagulant may prevent agglutination.

The leucocyte agglutination is detected by unstable leucocyte count, abnormal WBC histograms and presence of clusters or clumps of leucocytes on the PBS [1]. A demonstrated increase in the VCS parameters (mean channels of cell volume, conductivity, and light scatter),

such as MNV (mean channel of neutrophil volume) and especially NDW (neutrophil volume distribution width), may be used as an adjunct indicator for neutrophil agglutination [10]. In many but not all instances flagging and/or an abnormal WBC differential scattergram will alert the operator or technician.

Conclusion

With the increasing application of automated analyzers for routine hematological procedures, the possibility of spurious results has to be kept in mind. Unexplained neutropenia or leucopenia on automated hematology analyzers should always be confirmed by a blood smear examination, which can detect leucoagglutination.

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