Laboratory Case Report

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A case report of pseudoleukopenia: playing hide-and-seek

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Abstract

Objectives: Routine blood examination, one of the most commonly performed tests in clinical laboratories, directly reflects the overall state of the body such as inflammation, anemia, and thrombocytopenia. The accuracy of these indicators by tests may be perturbed by various factors including anticoagulants, antibodies, and temperatures. Pseudoleukopenia caused by leukoagglutination was rarely described in the literature.
Case presentation: We report a rare and unusual pseudoleukopenia case of a 75-year-old female with a stroke. Blood samples from the patient were collected using different anticoagulants and determined the hematologic parameters and blood smears. We observed the extent of leukocyte aggregation at different anticoagulants or temperatures. The intensity of leukoagglutination was attenuated after incubating at 37 °C for 30 min. After anti-infection treatment and symptomatic treatment, the leukoagglutination of the patient gradually weakened.
Conclusions: We have found the reason for the pseudoleukopenia and the leukocyte aggregation phenomenon may vary with disease progression.

Keywords: aggregation; EDTA; leukoagglutination; pseudoleukopenia.

Introduction

With the widespread application of the automated haematology analyzers (HA) in the clinical laboratory, a timely and accurate detection has become particularly important. Complete blood count (CBC) is one of the most prevalent blood tests, which can provide haematology parameters, such as erythrocytes, leukocytes, and platelet counts [1]. Platelet agglutination which falsely results in lowering platelet count and elevating white cell count has been well-known by laboratory professionals and gained particular attention in daily work [2–4]. Likewise, a similar phenomenon has also been found in white blood cells (WBC) or even rarer. It was first reported that spurious leukopenia was caused by neutrophil aggregation in 1983 [5]. Since then, a small number of cases with leucoagglutination have been reported [6–8]. Herein, we report a case of spurious WBC count.

Case presentation

A 75-year-old female patient with a history of diabetes mellitus and hypertension was admitted to the hospital because of sudden syncope accompanied by left-sided limb impairment. Subsequently, she developed progressive consciousness disturbance, atrial fibrillation and gastrointestinal bleeding. After admission, she received symptomatic treatment. On examination, she was afebrile, with mild disorders of consciousness but vitally stable. While neurological examination showed the muscle strength of her left upper and lower extremities was at grade I and grade II respectively, and a positive Babinski sign on the left side. Muscle strength in the right was normal.

The blood routine tests at admission were as follows: White blood cell count 15.60×10^9/L, neutrophils 92.1%, lymphocytes 5.2%, red blood cell count 3.31×10^{12}/L, and hemoglobin 90 g/L. No autoantibodies were detected and the fecal occult blood test was negative. The results of laboratory examinations are presented in the Supplementary Material. After admission, the patient was administered the infusion of human albumin solution and

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suspended red blood cells due to gastrointestinal bleeding and blood coagulation disorder.

On day 43rd following hospital admission, it was observed that there were significant differences in the WBC counts and classification between Beckman DxH-800 Coulter and Mindray BC-5180 for the blood sample (Table 1). Interestingly, 'NE blast' was warned by the Coulter analyzer at that time, but no warning cue at BC-5180. No aggregation of leukocytes was detected on two haematology analyzers. Microscopically, clumps with leukocytes as well as streets with several hundreds of leukocytes were demonstrated. These aggregates were found to be composed of a variable number of neutrophils, some of which contain monocytes or lymphocytes (Figure 1A–C). After incubation at 37 °C for 30 min, we repeated the CBC on instruments and a peripheral blood smear. A marked increase in total leukocyte count of the incubated sample in Beckman DxH-800 Coulter, but no major change in BC-5180 was evident (Table 1). Meanwhile, a terminal blood sample collected at the bedside was used to measure CBC and prepare blood smears stained with Wright–Giemsa stain. The agglutinated phenomena with slightly lower intensity were also observed in the capillary blood specimen and the incubated sample (Figure 1D–F). Five days later, the blood routine tests were conducted again which revealed similar results (Table 2). In particular, leukoagglutination was also observed in blood anticoagulated with citrate (Figure 2A, B). Half a month later, the agglutination phenomenon was attenuated in EDTA or citrate-anticoagulated samples (Figure 2C–E). There were no significant differences in leukocyte count and classification on two haematology analyzers (Table 3). Given the poor prognosis of the patient, her family chose to forgo further therapy and she was discharged from the hospital.

Table 1: The first detection of routine blood tests.

	Beckman DxH-800 coulter		Mindray BC-5180		Mindray BC-5180
Type	EDTA anticoagulation tube				Terminal blood sample
Temperature, °C	22	37	22	37	22
WBC, $\times 10^9$/L	3.6	7.3	6.17	5.9	11.07
NE%	30.89	60.89	60.9	61.2	52.8
LY%	56.23	30.06	32.2	31.8	42.3
MO%	8.16	6.47	4.8	4.4	4.5
EO%	3.60	1.87	1.8	2.2	0.4
BA%	1.12	0.71	0.3	0.4	0

Discussion

The rare aggregation phenomenon has been reported worldwide sporadically over the last several decades, but its etiology remains unknown [9–11]. Ethylenediaminetetraacetic acid (EDTA) can induce platelet aggregation, which is

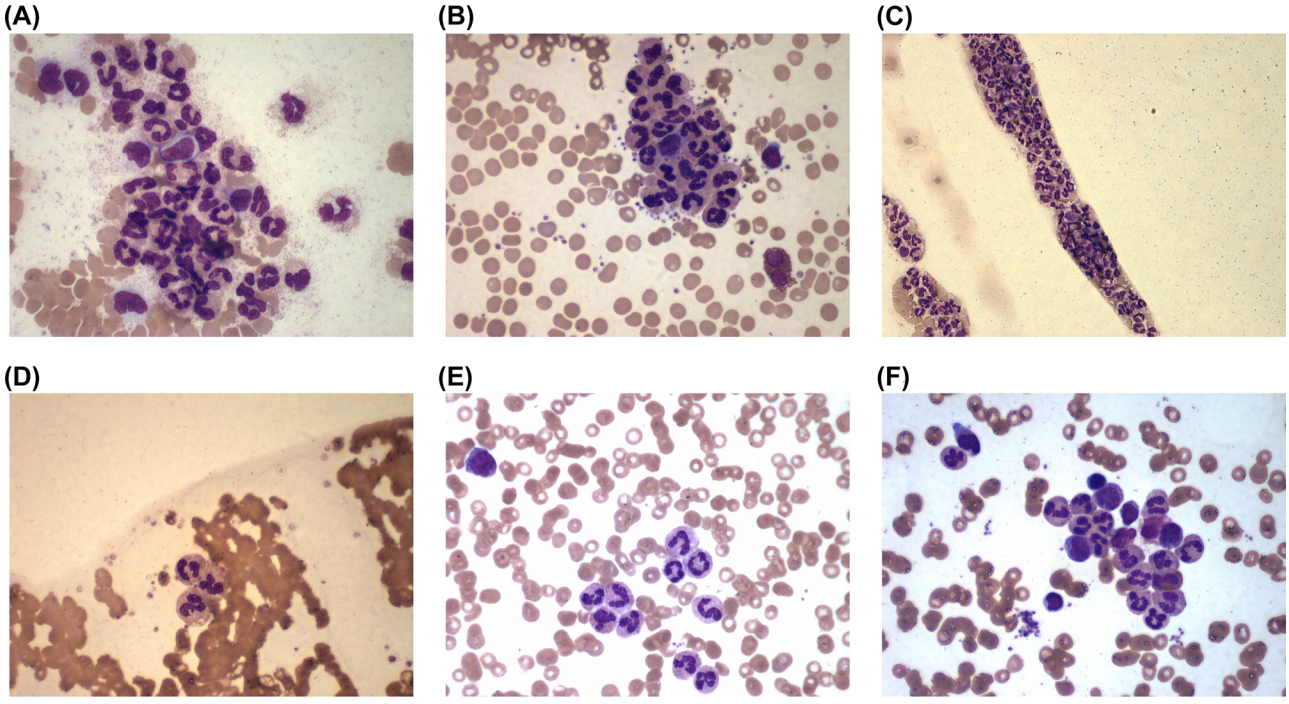

Figure 1: The agglutination phenomenon of samples under various temperature conditions. (A–C) Blood smears of an EDTA sample. (D) Blood smears of an EDTA sample after incubation at 37 °C for 30 min. (E, F) Blood smears of a terminal blood sample. Notably, the larger size of aggregates in EDTA blood compared to later other smears. Wright–Giemsa stain (C: ×400; A–F: ×1,000).

Table 2: Subsequent detection of routine blood tests.

	Beckman DxH-800 coulter		Mindray BC-5180		Mindray BC-5180
Type	**EDTA**	**Sodium citrate**	**EDTA**	**Sodium citrate**	**Terminal blood sample**
WBC, $\times 10^9$/L	6.4	10.7	13.79	13.62	15.34
NE%	68.23	79.84	81.4	77.5	63.8
LY%	24.98	16.46	16.8	19.0	32.1
MO%	6.52	3.16	1.5	2.9	3.9
EO%	0.11	0.16	0.2	0.4	0.2
BA%	0.16	0.38	0.1	0.2	0

All tests were performed at room temperature.

EDTA-dependent pseudothrombocytopenia (PTCP) [12]. It has been reported that various diseases are associated with EDTA-induced platelet agglutination and leukocyte agglutination, such as malignant tumors, liver diseases, autoimmune diseases, or infections [10, 13].

Such a phenomenon has been previously reported for an EDTA-dependent IgM antibody [14]. In this case report, the leukocyte aggregation has been not restricted to anticoagulated whole blood, being observed also in the finger-prick blood samples. This was in accordance with the phenomenon observed by Claviez et al. [15]. In particular, leukocyte aggregation may show different degrees in different anticoagulants with stronger aggregation in EDTA than that in sodium citrate. We cannot determine whether EDTA was solely responsible for the difference in the degree of leukocyte aggregation due to the lack of antibody detection in the case. But the possible reason for bigger clumps of leukocyte aggregation may be EDTA facilitated strong aggregation of leukocyte clumps. Concomitantly, the leukocyte aggregates in the blood at room temperature were shown to yield larger sizes compared to the incubation at 37 °C (Figure 1A–D). This phenomenon was reported previously, but the mechanism has not been defined [16]. Although no formal laboratory proof of antibodies in serum could be achieved, the changes in the classification and counts of leukocytes with Beckman DxH-800 Coulter after incubating indicate that the aggregation may be partially mediated through cold agglutinins.

The large aggregates (several hundreds of leukocytes) seen on blood smears might be overlooked in HA because these aggregates cannot flow into the counting aperture. The counting pore diameters on both HAs are 100 µm, which are much less than the diameter of aggregates containing dozens or hundreds of leukocytes. The different results of classification and counts of leukocytes for the same tube on Beckman DxH-800 Coulter and Mindray BC-5180 are rarely reported (Tables 1, 2). The significant discrepancies in results between the two HAs (Coulter S-Plus IV and Sysmex E-5000) were observed previously only by Rohr et al. [10]. On the same counting aperture basis, this discrepancy could be probably attributed to the

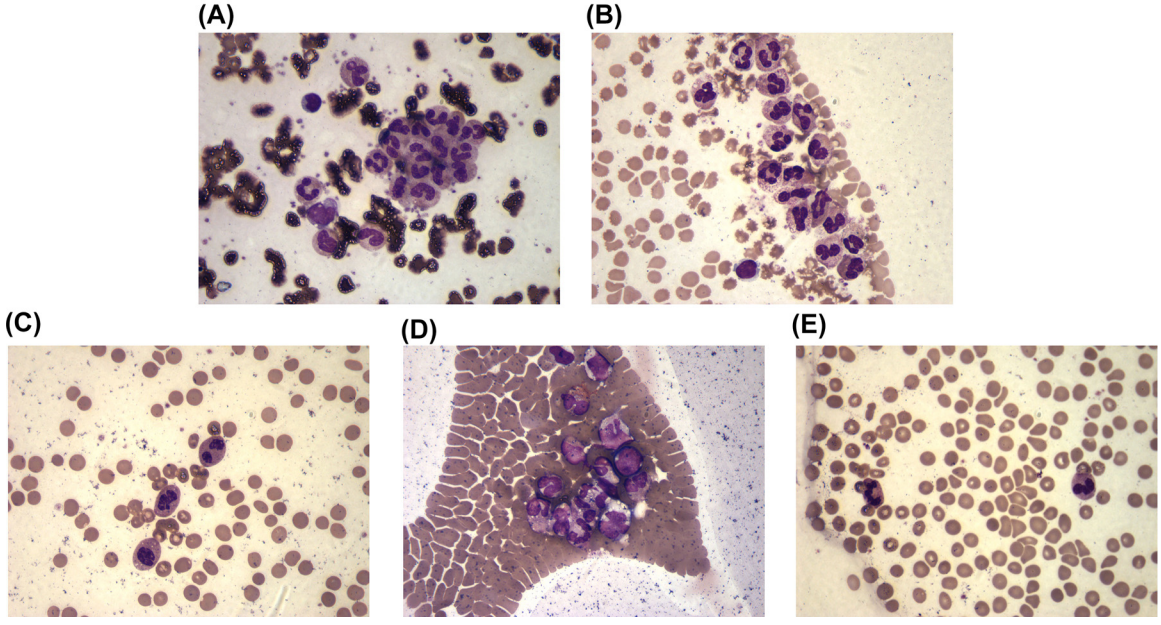

Figure 2: The agglutination phenomenon of samples from different anticoagulants at various time points. (A) Blood smears of an EDTA sample. (B) Blood smears of a sample anticoagulated with sodium citrate. (A, B) Blood samples were taken five days after the most recent test. (C) Blood smears of an EDTA sample. (D) Blood smears of a sample anticoagulated with sodium citrate. (E) Blood smears of a terminal blood sample. (C–E) Blood samples were taken after half a month. Meanwhile, the aggregation phenomenon was reduced. Wright–Giemsa stain (×1,000).

Table 3: The detection of routine blood tests after half a month.

	Beckman DxH-800 coulter	Mindray BC-5180	Mindray BC-5180
Type	**EDTA anticoagulation tube**		**Terminal blood sample**
WBC, $\times 10^9$/L	5.1	5.39	5.07
NE%	55.02	55.4	60.9
LY%	33.76	36.7	32.5
MO%	8.96	6.2	4.7
EO%	1.73	1.6	1.9
BA%	0.53	0.1	0

All tests were performed at room temperature.

different lysing agents which have the depolymerization effect differently for aggregates of varied sizes.

From day 34 after admission, the patient had gastrointestinal bleeding, as well as abnormal coagulation-related indicators (PT: 54.9 s, D-dimer: 32500 ng/mL). The deterioration of the patient may also have contributed to induce aggregation of leukocytes. There might be abnormal proteins or antibodies in the blood, which could trigger leukocyte aggregation. We propose that the deterioration of the patient may be contributed to induce the aggregation of leukocytes. Reexaminations of blood routine after half a month of treatment already have shown that there were no obvious differences between the two different HAs or between anticoagulated blood and non-anticoagulated terminal blood. The last measurement suggests the phenomenon in the patient may be transient and weakens until it eventually disappears over time, as in such published cases [7, 10, 16, 17]. As the disease progresses, the patient developed hypoproteinemia (ALB: 22 g/L) despite supplements of albumin. Low albumin levels may lead to an immunosuppressed condition [18]. We propose that immunosuppression states may be one of the reasons for the disappearance of leukocyte aggregation. Regrettably, due to limited laboratory facilities and inadequate testing capacity, we couldn't further confirm the exact reason for leukocyte aggregation. Nevertheless, awareness of leukocyte aggregation for every laboratory personnel can assist in distinguishing pseudoleukopenia thus preventing misdiagnosis and inappropriate treatment in the clinic.

Conclusions

In conclusion, we found an uncommon coincidence of leukocyte aggregation, which is likely to be a multifactorial phenomenon that varies with disease progression.

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References

1. Kuan DH, Wu CC, Su WY, Huang NT. A microfluidic device for simultaneous extraction of plasma, red blood cells, and on-chip white blood cell trapping. Sci Rep 2018;8:15345.
2. Baele G, De Paepe M, Vermeulen A. Pseudothrombocytopenia. Acta Clin Belg 1978;33:303–7.
3. Zupańska B, Maślanka K. Analysis of 15 cases with platelet EDTA-dependent antibodies. Acta Haematol Pol 1995;26:361–5.
4. Lee AC. Pseudothrombocytopenia: what every clinician should know. Pediatr Neonatol 2021;62:218–9.
5. Guibaud S, Plumet-Leger A, Frobert Y. Transient neutrophil aggregation in a patient with infectious mononucleosis. Am J Clin Pathol 1983;80:883–4.
6. Epstein HD, Kruskall MS. Spurious leukopenia due to in vitro granulocyte aggregation. Am J Clin Pathol 1988;89:652–5.
7. Sultan S, Irfan SM. In vitro leukoagglutination: a rare hematological cause of spurious leukopenia. Acta Med Iran 2017; 55:408–10.
8. Bai Z, Zhu X, Yun C, Bao Y. Plasma cell leukemia characterized by EDTA-dependent plasma cell agglutination: a case report. Ann Palliat Med 2020;9:2330–2.
9. Walradth EA. Lymphocyte aggregation in low-grade B-cell lymphoma. Lab Med 2021;52:e133–6.
10. Rohr LR, Rivers FM. Spurious automated leukopenia due to in vitro granulocyte aggregation. Am J Clin Pathol 1990;93:572–4.
11. Moraglio D, Banfi G, Arnelli A. Association of pseudothrombocytopenia and pseudoleukopenia: evidence for different pathogenic mechanisms. Scand J Clin Lab Invest 1994;54: 257–65.
12. Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. Clin Chem Lab Med 2012;50:1281–5.
13. Berkman N, Michaeli Y, Or R, Eldor A. EDTA-dependent pseudothrombocytopenia: a clinical study of 18 patients and a review of the literature. Am J Hematol 1991;36:195–201.
14. Hillyer CD, Knopf AN, Berkman EM. EDTA-dependent leukoagglutination. Am J Clin Pathol 1990;94:458–61.
15. Claviez A, Horst HA, Santer R, Suttorp M. Neutrophil aggregates in a 13-year-old girl: a rare hematological phenomenon. Ann Hematol 2003; 82:251–3.
16. Shelton JB Jr., Frank IN. Splenic B cell lymphoma with lymphocyte clusters in peripheral blood smears. J Clin Pathol 2000;53: 228–30.

17. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes. Int J Lab Hematol 2007;29:21–41.
18. Wang Y, Chen L, Zhang B, Song W, Zhou G, Xie L, et al. Pretreatment inflammatory-nutritional biomarkers predict responses to neoadjuvant chemoradiotherapy and survival in locally advanced rectal cancer. Front Oncol 2021;11:479–90.

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