

Letter to the Editor

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Remarkable pseudoleucocytosis induced by mild cryoglobulinemia

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To the Editor,

We recently met a special case during routine blood examination. A 56-year-old man was diagnosed with B-cell lymphoproliferative disorder (Waldenström's macroglobulinemia, WM) and admitted to hospital for treatment. Here are his laboratory examination results on admission. Complete blood cell count (CBC) results measured using the Coulter DxH800 automatic hematology analyzer ("Coulter" in short, Beckman Coulter, Brea, CA, USA) were: WBC $122.83 \times 10^9/L$ (ref. $3.50\text{--}9.50 \times 10^9/L$), RBC $3.25 \times 10^{12}/L$ (ref. $3.80\text{--}5.10 \times 10^{12}/L$), Hb 94 g/L (ref. 130–175 g/L), and PLT $191 \times 10^9/L$ (ref. $125\text{--}350 \times 10^9/L$). The patient was with normocytic normochromic anemia, which was the manifestation of WM. The WBC histogram showed a decreasing trend (Figure 1A) and the analyzer alarm indicated the presence of nucleated red blood cells (NRBCs). Peripheral smear prepared by the Coulter DxH Slidemaker Stainer (Beckman Coulter, Brea, CA, USA) indicated normal WBC count and none of NRBCs (Figure 1D). However, a large amount of irregular blue-gray lump-like deposits were observed on the smear prepared manually (Figure 1E). The specimen was immediately assayed by Coulter after the blood was heated to 37 °C for 30 min. The results were as follows: WBC $6.80 \times 10^9/L$, RBC $3.20 \times 10^{12}/L$, Hb 99 g/L, and PLT $166 \times 10^9/L$. The WBC histogram of Coulter showed a normal distribution without any

alarms (Figure 1B). Microscopic examination showed that these blue-gray deposits disappeared (Figure 1F). Other laboratory tests included: (1) Serum protein concentration: total protein 44.6 g/L (ref. 65.0–85.0 g/L), albumin 28.6 g/L (ref. 40.0–55.0 g/L), and globulin 16.0 g/L (ref. 20.0–40.0 g/L). (2) Immunoglobulin assay: IgG 6.21 g/L (ref. 7.00–16.00 g/L), IgA 0.41 g/L (ref. 0.70–4.00 g/L), and IgM 2.86 g/L (ref. 0.40–2.30 g/L). (3) Identification of cryoglobulin: cryocrit was 0.6% (ref. <0.4%), and serum immunofixation electrophoresis showed light IgM-kappa bands. (4) Renal function tests and renal biopsy: urea nitrogen was 15.04 mmol/L (ref. 2.1–7.2 mmol/L) and creatinine was 748.4 $\mu\text{mol}/L$ (ref. 44–132 $\mu\text{mol}/L$). Because of the worse renal function, a renal biopsy was performed. The pathology results and immunofluorescence findings confirmed type I cryoglobulinemia.

Cryoglobulinemia is defined as the persistent presence of cryoglobulins in serum. The presence of cryoglobulin can lead to erroneous WBC counts and PLT counts in many instances [1–5]. As listed in Table 1, the influence of cryoglobulin on CBC is pseudoleucocytosis and pseudothrombocytosis. Only a few cases were pseudothrombocytosis, while most cases were pseudoleucocytosis. The highest fold recorded for pseudoleucocytosis was reported by Geara et al. [2], which was 8.0. Therefore, to the best of our knowledge, the pseudoleucocytosis of this case is the most prominent with an 18-fold difference. CBC detection mechanism of Coulter series counters is the electrical impedance method. The increased resistances are sensed by the instrument when cells of various sizes flow through an aperture where there is an electrical current. We analyzed the possible reasons for the 18-fold increase in WBC count. Firstly, IgM was 2.86 g/L, just slightly higher than 2.30 g/L. In the WBC channel of Coulter, the whole blood is 1:251 diluted, which means the cryoglobulin concentration will become much lower. Therefore, cryoglobulin itself unlikely causes such a remarkable pseudoleucocytosis. Secondly, the Coulter alarm indicated the presence of NRBCs (not "RBC fragments"). Under microscope, however, no NRBC was observed and the RBCs were surrounded by

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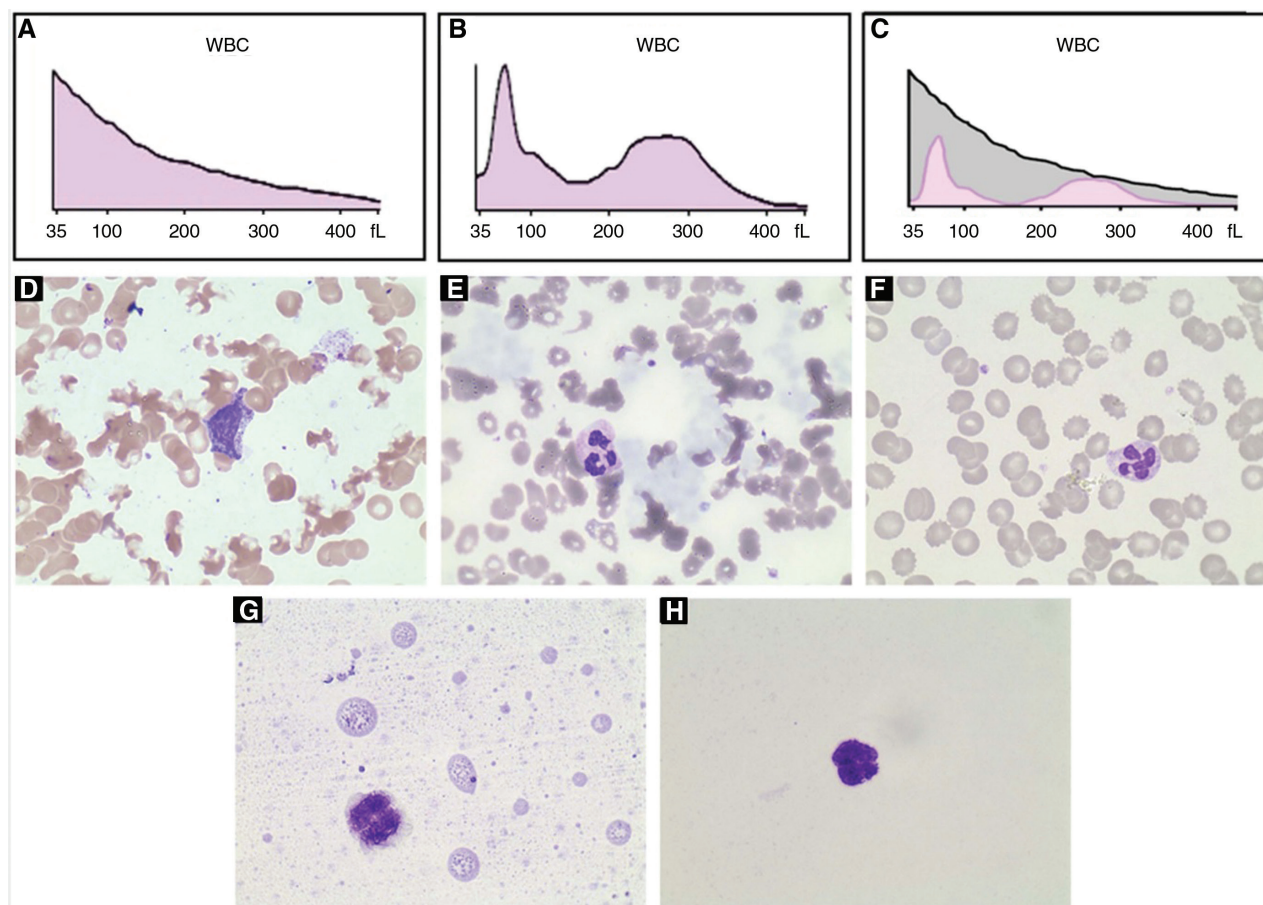


Figure 1: WBC histograms and peripheral blood smears of the patient's sample.

(A–C) WBC histograms. (A) At room temperature. (B) 37 °C for 30 min. (C) The merged image. (D–H) Blood smear (Wright-Giemsa stain; ×1000). (D) Prepared by the Coulter DxH Slidemaker Stainer at room temperature. (E) Prepared manually at room temperature. (F) Prepared manually after the blood was heated to 37 °C for 30 min. (G) and (H) The additional experiment. (G) This case. (H) Normal control.

Table 1: Influence of cryoglobulin on the CBC test by different hematology analyzers.

Publications	Analyzer	WBC count, $\times 10^9/L$			PLT count, $\times 10^9/L$		
		RT	37 °C	Folds (=RT/37 °C)	RT	37 °C	Folds (=RT/37 °C)
Fohlen-Walter et al. [1]	Bayer Technicon H*2	14.94	4.30	3.5	213	234	0.9
	Bayer Technicon H*2	12.61	11.51	1.1	906	112	8.1
	Bayer Technicon H*2	3.08	3.02	1.0	173	112	1.5
	Bayer Technicon H*2	7.77	6.23	1.2	272	269	1.0
	Coulter STKS	9.3	4.4	2.1	213	204	1.0
	Coulter STKS	15.3	10.9	1.4	128	85	1.5
	Coulter STKS	5.4	2.8	1.9	106	99	1.1
Geara et al. [2]	Coulter GenS	96	12	8.0	No	No	
Abela et al. [3]	No	54.0	8.4	6.4	No	No	
Haeney et al. [4]	Model S Coulter Counter	11.5	6.3	1.8	No	No	
	Model S Coulter Counter	14.9	9.5	1.6	No	No	
Taft et al. [5]	Model S Coulter Counter	7.6	5.0	1.5	No	No	
This case	Coulter DxH800	122.83	6.80	18.1	191	166	1.2

No, not done or not mentioned; RT, room temperature; 37 °C, after 30 min at 37 °C.

cryoglobulin. Therefore, the speculation of pseudoleucocytosis is as follows: RBCs and IgM-kappa cryoglobulin form lump-like RBC/cryoglobulin complexes. When the blood goes through the WBC channel, lump-like RBC/cryoglobulin complexes are degraded by lysis solution into fragmented RBC/cryoglobulin complexes of different sizes. As a consequence, these fragmented complexes derived from 10^{12} -magnitude RBCs produce such a remarkable spurious increase in the WBC count. These fragmented complexes also lead to the specific histogram in Figure 1A, which overlaps the normal histogram of WBCs (Figure 1C is the merged image). And those fragmented complexes with size near to NRBC are recognized as NRBCs. This speculation later is confirmed by an additional experiment. We mimicked the mixture in the WBC channel according to the manufacturer's instruction (6 mL dilution solution, 28 μ L whole blood, and 1.08 mL lysis solution), centrifuged the mixture at 500 g for 5 min, and prepared a smear using the cellular layer. A lot of fragmented RBC/cryoglobulin complexes can be found (Figure 1G).

Moreover, no cryoglobulin can be seen on the slide prepared by the Coulter DxH Slidemaker Stainer, which is due to a two-step heating process of the instrument settings. Therefore, we recommend manual preparation or automatic slide maker without the heating step to find clues of cryoglobulin on peripheral smears.

All the experiments were conducted after the protocol was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. Written

informed consent for the use of the clinical samples was obtained from the patient.

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