Unusual morphological cryoglobulin manifestations on blood and bone marrow smears

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Case report

A 66-year-old female presented with a 3 years history of multiple ulcerations on the lower extremities of both legs. The diagnosis of necrotic angiodermitis had been previously proposed. The skin lesions were numerous (6 on right side and 7 on left side), purpuric, evolutive and painful, with width ranging from 0.5 to 4.5 centimeters. No adenopathy, neither liver or spleen enlargement was found. Deep vein thrombosis was ruled out by ultrasonography. At admission, blood cell count anomalies were observed (Table 1), in particular high platelet count (945×10^9/L). The blood cell analyzer (Bayer Advia 120, Tarrytown, NY, USA) indicated a high percentage of large platelets, red blood cell fragments and ghosts. The blood smear showed a peculiar aspect of poïkilocytosis, with multiple loss of red blood cell periphery (*bite cell*, Figure 1). Staining the blood film with phloxine B revealed micro-aggregates of pink amorpheous particles between red cells (Figure 2) suggesting the presence of cryoglobulin precipitates. No basophilic inclusion body in the neutrophils was observed. A serum prepared from whole blood at 37°C demonstrated protein precipitation while cooling to room temperature. The protein was identified as a mixed cryoglobulin (type 2, IgG+IgM), with a monoclonal component IgM Kappa. The clotting screen and the thrombophilic explorations were normal (proteins C and S, antithrombin 3, Leiden factor), apart an anticardiolipn IgG. A cold agglutinin was excluded. Skin biopsy demonstrated capillaries thrombosis. A bone marrow aspiration was performed showing 22% lympho-plasmocytes and 3% plasma cells. Extracellular deposits were observed again. Moreover, one part of the plasma cells contained dust -like cytoplasmic inclusions (Figure 3) and some of the macrophages had phagocytized amorpheous particles (Figure 4). Diseases often associated with cryoglobulins were unsuccessfully investigated (lymphoma, multiple myeloma, systemic lupus erythematosus, carcinoma, glomerulonephritis, chronic hepatitis...). In the absence of any apparent relevant disease, the significance of the cryoglobulinemia remains currently unclear. An autoimmune disease is still questionable.

Discussion

We reported on a patient with skin lesions due to a previously misdiagnosed cryoglobulinemia, first revealed by an artefactual thrombocytosis.

Cryoglobulins are immunoglobulins or immune complexes including immunoglobulins which reversibly precipitate upon exposure to cold temperature and redissolve at high temperature. The presence of chronic leg ulcers in the supramalleolar region in the absence of severe stasis dermatitis or arterial vasculopathy can reveal systemic diseases such as connective tissue diseases, infections or cryoglobulinemia.[1] Nevertheless, recognition of the haematologic abnormalities associated with cryoglobulins may be the first clue leading to the diagnosis of cryoglobulinemia and eventually to its underlying cause.

Automated cell counters may mistake the precipitated cryoglobulins for blood cell when the size of the precipitates falls within the range of the aperture through which cells in suspension are drawn, resulting in spuriously elevated white blood cells or platelets counts.[2] In our case,

Figure 1. Artefactual poïkilocytosis with bitten-like red blood cells (blood smear, x400, May-Grundwald-Giemsa).

Figure 2. Aggregates of amorpheous particles (blood smear, x1000, phloxine B).

Figure 3. Inclusion bodies in a plasma cell and amorpheous aggregates* (bone marrow, x1000, May-Grundwald-Giemsa).

Figure 4. Phagocytosis of amorpheous particles by macrophages and extracellular deposits* (bone marrow, x1000, May-Grundwald-Giemsa).

Table 1. Patient's data.

		May, 2003				August, 2003		
		7	13	19		5		
full blood counter			Bayer Advia 120			Coulter T540	Bayer Advia 120	
temperature		room	room	room	room	room	room	37°C, 30 minutes
haemoglobin	(g/dl)	10.5	10.7	10.2	10.5	11.1	11.1	11.1
mean cell volume	(fl)	89.2	91	89.9	88.4	91.6	92.1	92.8
leukocytes (basophil/lobularity channel*)	($x10^9$/l)	4.11	4.22	3.60	3.80	131.7	3.85	3.94
leukocytes (peroxydase channel*)		4.00	3.95	3.48	3.88		3.76	3.68
platelets (raw data)	($x10^9$/l)	945	391	1943	2049	1437	1163	353
mean platelet volume	(fl)	17.1	10.5	13.4	13.6		17.9	6.2

the platelet measurements were mainly affected by the presence of the cryoglobulins, associated with alarms for the platelet and erythrocytic series. The *true* platelet count was likely comprise between 300-400×10^9/L in agreement with ancient patient's data and the drop to 353×10^9/L after warming. For white blood cells, two cell counts are obtained with the Bayer technology: complete cells are measured with the peroxidase channel whereas only cell nuclei are detected after membrane lysis with the basophil/lobularity channel. As no significant difference was observed between the two counts, one can state that the white cell counts were not affected by the cryoglobulinemia. By contrast, an interference occured with an impedance method (Table 1).

We reported previously on the variable morphology of cryoglobulins in fresh blood drop and on stained blood smear.[3] Cryoglobulins could be seen in the neutrophils or monocytes as a single or multiple round inclusions sometimes displacing the nucleus, corresponding to an in vitro phagocytosis.[4,5] Some of these pictures were close to the macrophages appearence of our case. Cryoglobulins should be distinguished from other exogenous neutrophils inclusions, as abnormal mucopolysaccharides, lupus erythematosus (LE) cells, and fragments of red cells ingested in haemolytic anaemias.[6] Only a few reports on the manifestations of cryoglobulins on blood smears have been reported, so far as May-Grünwald Giemsa stained blood smears are usually normal. Cryoglobulin may sometimes become visible as extra-cellular material, forming small grayish or pinkish precipitates.[7] We first time observed (and, to our knowledge, report) the very peculiar aspect of our case, with unvisible droplets distorting the adjacent red blood cells.

Inclusions were described in plasma cells in normal and reactive conditions (mainly in multiple myeloma and lymphoproliferative disorders.[8] Crystallisation has seldom been reported in cryoglobulinemia, with variing morphology.[9] We suggest the dust-like inclusions of the plasma cells of our patient likely to be immunoglobulins,[10] and, possibly one component of the cryoglobulinemia. To conclude, we would like to point out that laboratory recognition of the cryoglobulins is important in order to correct factitious results with automated blood cell counters. Moreover cryoglobulin-induced blood smear artefacts may be the first factor prompting the assessment for cryoglobulinemia and, possibly, the diagnosis of an underlying cause.

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