Erroneous values for the total white cell count and ESR in patients with cryoglobulinaemia

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SYNOPSIS Temperature dependent fluctuations in the erythrocyte sedimentation rate were noted during the assessment of one patient with symptomatic cryoglobulinaemia. Two patients with this condition were found to have erroneously high leucocyte counts when estimated by the Model Coulter Counter. The artefact was directly related to the degree of exposure to cold before the estimation was performed and appeared to be due to the formation of microaggregates of cryoglobulin and fibrinogen.

Cryoglobulins are cold-precipitable immunoglobulins which redissolve at warm temperatures. These serum proteins have been described in a variety of clinical conditions in man (Ritzman and Levin, 1961), but particularly in individuals with various infections, autoimmune and neoplastic disorders (Mackay *et al*, 1956; Meltzer *et al*, 1966; Barnett *et al*, 1970).

In assessing one patient with symptomatic cryoglobulinaemia, fluctuations in the erythrocyte sedimentation rate were noted. In addition, the same patient demonstrated erroneously high leucocyte counts when the Model S Coulter Counter was used but normal values when counting was performed by hand. The variations in ESR and WBC counts were found to be temperature dependent and thus susceptible to environmental conditions. A further patient with symptomatic cryoglobulinaemia also showed similar changes in leucocyte counts.

Case reports

CASE 1

J.P., a 60-year-old car worker, presented in September 1973 with a two-year history of recurrent chest infections and a six-month story of Raynaud's phenomenon affecting his hands, feet, and face. Preliminary investigations at a different hospital had been passed as normal. In particular, the erythrocyte sedimentation rate was 20 mm fall in one hour. He was discharged from follow-up but because of the persistence of symptoms he was referred to this department by his family doctor. Physical examination was non-contributory. Rou-

tine investigation showed marked fluctuations in ESR (20-120 mm fall/1 hour) and total white cell count (6.18×10^9 cells/l) on a day-to-day basis. He was subsequently found to have a monoclonal IgG paraproteinaemia with cryoglobulinaemia. The mean quantitative serum IgG level was 24 g/l (normal 6-16 g/l); mean IgA 0.29 g/l (0.75-5.2); IgM 0.08 g/l (0.3-1.8). The serum cryoglobulin consisted of monoclonal IgG in a concentration of 21.4 g/l. Increased numbers of atypical IgG staining plasma cells were present in the bone marrow and a pathological fracture of the right third rib was demonstrated radiologically. The patient was considered to have multiple myelomatosis with cryoglobulinaemia. The disturbing variation in results obtained for the ESR and leucocyte counts prompted this study of the effect of temperature on these parameters in the patient.

CASE 2

B.S., a 45-year-old woman of Russian extraction presented in August 1974 with the nephrotic syndrome. In the preceding 15 years she had experienced two episodes of an intermittent purpuric erythematous papular eruption on the legs with a bilateral arthropathy affecting the knees and ankles. A skin biopsy was compatible with the diagnosis of allergic vasculitis. Renal function was normal at this time but the Rose-Waaler test was positive at a titre of 1/128. A trace of cryoglobulin was found in the serum six years before the present admission. She responded to systemic steroids which were discontinued after 12 months.

In 1974 she presented with ankle oedema of eight months' duration. She was found to have a nephrotic syndrome with non-selective massive proteinuria of

10-15 g per 24 hours. The creatinine clearance was initially 70 ml/min but later rose with treatment to over 100 ml/min. The Rose-Waaler was positive at a titre of 1/128. The serum immunoglobulins were abnormal: IgG 2.08 g/l (normal 6-16); IgA 0.83 g/l (0.75-5.2); IgM 2.24 g/l (0.3-1.8). Complement levels were low: C3 was 0.51 g/l (normal 0.78-1.61) and C4 was remarkably low at 0.01 g/l (0.15-0.45); Clq was also low. The serum contained a mixed cryoglobulinaemia composed of monoclonal IgM, type kappa (mean concentration 2.8 g/l) and IgG (mean concentration 1.5 g/l). Increased numbers of plasma cells staining with IgM and IgG and normal numbers of IgA staining plasma cells were seen on bone marrow immunofluorescence. A skin biopsy revealed scattered deposition of coarse aggregates of IgM and IgG and, to a very small extent, of complement. These aggregates were related to blood vessels. The histology of the renal biopsy was that of membranoproliferative glomerulonephritis, and on immunofluorescence granular deposits of IgG and IgM were seen on the epithelial basement membrane. Complement staining was remarkably faint. It thus appears that this patient has a 'soluble immune complex disease' due to the mixed cryoglobulinaemia with cutaneous vasculitis, an arthropathy, and membranoproliferative glomerulonephritis producing a nephrotic syndrome. Routine assessment of this patient also revealed fluctuations in leucocyte counts seen previously in case 1.

Methods

Blood was drawn in prewarmed (37°C) tubes containing potassium EDTA and maintained at 37°C. Thereafter blood was divided into four samples; one sample was kept at 37°C and the others were cooled to 20°C, 12°C, or 4°C. All samples were maintained at these temperatures for one hour before the estimation of erythrocyte sedimentation rate and white cell count. The ESR was then simultaneously estimated for each sample at 20°C in a 200 mm column. The Westergren method was used with sodium citrate 3.8% solution to make a 20% dilution of blood. The reading was taken at one hour.

In the two patients with cryoglobulinaemia, total leucocyte counts were also estimated on the four samples using a Model S Coulter Counter.

Results

ERYTHROCYTE SEDIMENTATION RATES (table I)

Group I: Normal controls

Twenty normal controls were evaluated. These con-

sisted of members of staff and informed hospital inpatients. The mean ESR for the group at one hour showed a gradual rise with increasing temperature up to 20°C, though all results were within the normal range. At 37°C the ESR was mildly increased.

Group	No. of estimations	Erythrocyte sedimentation rate (mm fall/hr)	Temperature (°C)			
			4	12	20	37
I Normal Control	20	Mean SEM ¹	6.2 1.0	7.6 1.4	9.9 2.0	16.1 2.6
II Non-specific increase	5	Mean SEM	39.0 7.6	75.2 12.1	90.6 10.6	107.6 10.1
III Cryoglobulinaemia	6	Mean SEM	5.6 1.4	17.0 5.4	40.8 12.7	111.8 8.3

Table I Effect of temperature variation on ESR in case 1, normal controls, and patients with other causes of an increased ESR

¹SEM = standard error of mean

Group II: Non-specific increase of ESR

Five patients with increased ESRs made up the group. An increase, in the terms of this study, was defined as an ESR in excess of 50 mm fall in one hour on three or more occasions. Cryoglobulinaemia was excluded in all cases. The ESR was grossly increased at all temperatures, the values at 4°C, 12°C, and 20°C being approximately 35%, 70%, and 85% respectively of the value obtained at 37°C.

Group III: Cryoglobulinaemia

Case 1 was studied on six occasions. In contrast to group II patients, the ESR at 4°C was normal and was only 4% of the ESR at 37°C. Similarly, at 12°C the ESR was only slightly increased and represented 15% of the value at 37°C. Even at 20°C the result was less than 40% of the ESR at 37°C and less than 50% of the corresponding values obtained in group II patients. However, at 37°C the ESR in the patient with cryoglobulinaemia was higher than the mean obtained for group II.

TOTAL WHITE CELL COUNTS (table II)

Total leucocyte counts were studied in the two patients with cryoglobulinaemia. The pattern obtained was similar in both cases. The counts were normal at 37°C using the Model S Coulter Counter. However, as the temperature was lowered, the total WBC counts rose to abnormal levels even after one hour at 20°C and reached values in excess of 20×10^9 cells/l after one hour at 4°C. The values obtained

by a manual method were normal at all temperatures in both patients.

Case	No. of estimations	Total leucocyte counts $\times 10^9/l$	Temperature ($^{\circ}C$)			
			4	12	20	37
1	6	Mean	24.0	13.3	11.5	6.3
		SEM ¹	4.1	1.6	1.3	1.1
2	10	Mean	21.9	17.7	14.9	9.5
		SEM	3.4	2.6	2.3	0.8

Table II Leucocyte counts obtained on the Model 'S' Coulter Counter in two patients with cryoglobulinaemia

¹SEM = standard error of mean

Discussion

Erythrocyte sedimentation rate is known to vary with room temperature (Rogers, 1946; Wartman, 1946), and Manley (1957) has prepared a nomogram for the correction of ESR by Westergren's method for temperature variations. The results obtained in this present study for groups I and II show a close correlation with the nomogram, the actual rise in ESR with temperature being similar to the predicted value. In contrast, the patient with cryoglobulinaemia gave results which failed to correlate with the correction graph, the readings at 20°C and 12°C underestimating the true ESR. In cryoglobulinaemic patients a false normal ESR may result if samples are allowed to stand at low temperatures for even a short time.

The same patient (case 1) also manifested considerable variation in the total white cell count on consecutive days when estimated by the Model S Coulter Counter. Manual leucocyte counts were repeatedly within the normal range. These spurious results have been shown to be temperature dependent (table II). Immediate estimations of the white cell count were normal on the automated counter but rose progressively if the samples were allowed to stand for one hour at low temperatures. If the samples, even after the cooling period, were warmed to 37°C before counting, or if warm diluent was used, then normal leucocyte counts were obtained. The conclusion is that the cryoglobulinaemia is responsible for this spurious leucocytosis. This was confirmed in the second patient with 'essential' cryoglobulinaemia. In case 2, it was found that if serum was separated from whole blood, and plasma separated from EDTA anticoagulated whole blood, then the leucocyte counts of both serum and plasma were similar if estimated immediately in the Coulter Counter. If samples were allowed to stand for one hour at lower temperatures, as described, then only the plasma samples showed a spurious leucocytosis

on the automated counter. This artefact was temperature dependent. It seems likely that the increased leucocyte counts may not be due to cryoglobulin alone but to microaggregates between cryoglobulin and fibrinogen. These findings confirm those of other workers who have shown spurious leucocytosis in cryoglobulinaemic patients (Emori *et al*, 1973). Taft *et al* (1973) described one patient with a spontaneously crystallizing paraproteinaemia in whom the leucocytosis was due to needle crystals of cryoglobulin appearing when blood was stored at 24°C but did not occur even after refrigeration to 4°C for 6 hours. This appeared to be related to the size and shape of the crystals produced.

A feature of chilled blood containing cryoglobulins is that platelet aggregation may occur sometimes to a striking degree (Cortellaro *et al*, 1975). However, the platelet aggregation described by Cortellaro *et al* is not reversible and is inhibited completely in the presence of EDTA. Visual and automated platelet counts at different temperatures have been performed on one patient (case 1). No significant difference was found in the values obtained at 4°C, 12°C, and 20°C. Although platelet aggregates, with their lipid membranes, are likely to interfere with the electric current in the automated counter, this does not appear to be the explanation in the cases reported.

For the estimation of leucocyte counts in patients known to have cryoglobulinaemia manual methods should be used or blood maintained at 37°C until estimated by the Model S Coulter Counter. Alternatively, warming the diluent entering the counter to 37°C eliminates the error. Inappropriately increased leucocyte counts should alert the clinician that a cryoglobulin may be present. In addition, in one of the patients described, the ESR showed fluctuations not seen in patients with increased sedimentation rates due to other causes. Environmental cooling of the blood before estimation of the ESR can produce values that may be interpreted as normal. This did occur in the case described, with subsequent delay in diagnosis and treatment.

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