The influence of paraproteinaemia on the Technicon automated platelet counter

P. C. HALL AND R. M. IBBOTSON

From the Department of Haematology, The London Hospital, London

SYNOPSIS In five patients whose serum contained a paraprotein it has been shown that platelet counts obtained using the Technicon AutoCounter are much too high. In two patients with IgG gammopathy the error is caused by some factor in the red cells lysed by the diluent solution 2M urea. In three patients with IgM gammopathy spurious platelet counts were produced by platelet-poor plasma when diluted with 2M urea. It is recommended that at the present time no patient known to have a paraprotein should have platelet counts performed on the AutoCounter.

The Technicon AutoCounter has an optical system which detects light impulses from particles illuminated in a flow cell. The light pulses are focused on a photomultiplier tube where they are converted to electrical pulses. An electrical counting device integrates these pulses into peaks which are displayed on a strip chart recorder. For the purposes of platelet counting, the machine samples whole blood which is then diluted and haemolysed with 2M urea. The peak heights correspond to the number of platelets counted per unit time.

During the routine use of this instrument, several recordings were observed to be off the scale. In accordance with the manufacturers' manual, dilutions were made of each particular sample. The results obtained from the diluted samples in no way agreed with an assessment of platelet numbers in the respective peripheral blood films. These anomalous findings are the subject of this preliminary communication.

Materials and Methods

The Technicon AutoCounter was used. The samples

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were initially haemolysed and diluted with 2 molar urea (Technicon Chemicals Co). Isoton (a modified Eagles solution) is supplied by Coulter Electronics Ltd.

Immunoglobulin assays were carried out on ID Agar plates at pH 8.2 after the method of Mancini, Carbonara, and Heremans (1965).

Four ml of blood was collected by a clean venepuncture into tubes containing 4 mg K₂EDTA. Where indicated platelet-rich plasma was obtained from this sample by centrifuging for 10 minutes at 100 g. Platelet-poor plasma was separated by centrifuging for 30 minutes at 2000 g.

Platelets were counted visually by phase contrast using an improved Neubauer chamber with diluting fluid consisting of 1% ammonium oxalate (Dacie and Lewis, 1970).

Results

During a six-month survey five patients were found whose platelet counts on the AutoCounter differed significantly from the appearances on film. Rowan, Allan, and Prescott (1972) suggested that the presence of paraprotein could interfere with auto-

Patient	Whole Blood	Whole Blood Counted after Dilution 1:3 and Count Corrected for Dilution	Platelet Count after Correction for PCV	
			Platelet-rich Plasma	Platelet-poor Plasma
J.A.	600 000	—	360 000	450 000
W.H.	> 750 000	1 020 000	750 000	750 000
J.H.	> 750 000	750 000	570 000	510 000
J.W.	> 750 000	330 000	225 000	0
L.L.	> 750 000	435 000	100 000	0

Table I Platelet count by Autocounter¹

¹Results expressed per μ l whole blood

Patient	Whole Blood	Platelet Count after Correction for PCV	
		Using Platelet-rich Plasma	Using Platelet-poor Plasma
J.A.	22 000	25 000	0
W.H.	470 000	600 000	0
J.H.	93 000	140 000	0
J.W.	220 000	189 000	0
L.L.	67 000	81 000	0

Table II Visual platelet count¹¹ Results expressed per μ l whole blood

mated platelet counting, and in all cases a monoclonal gammopathy was shown to be present.

Platelet-rich plasma was prepared by slow speed centrifugation and the samples processed through the AutoCounter. In two cases, J.W. and L.L., the machine counts of platelet-rich plasma (table I) agreed with the visual counts performed on the same samples (table II). However, the platelet counts on cases J.A., W.H., and J.H. showed little comparison.

Platelet-poor plasma prepared by high speed centrifugation, when processed through the AutoCounter, resulted in the generation of a platelet count in cases J.A., W.H., and J.H. Examination by microscopy of all platelet-poor specimens confirmed that they were particle free. This was substantiated by submitting the samples to the AutoCounter again after using Isoton as the diluent with the result that zero counts were produced.

Discussion

Rowan *et al* (1972) postulated that red cell stroma, when coated with the abnormal protein, could interfere with automated platelet counting. The results in two cases, J.W. and L.L., both with an IgG paraprotein (table III), supported this hypothesis as machine counts of platelet-rich plasma compared favourably with visual counts. Thus in these two cases the removal of red cells by prior centrifugation appeared to prevent interference with counting whereas haemolysis by the working reagent 2M urea failed to do so.

However, the counts of platelet-rich plasma on cases J.A., W.H., and J.M. when compared with visual counts did not show this correction. Platelet-poor plasma from these patients when processed

Patient	Monoclonal Protein (mg/100 ml)	Normal Range (mg/100 ml)
J.A.	1880	50-110
W.H.	1800	
J.H.	2880	
J.W.	2200	880-1500
L.L.	2100	

Table III Protein abnormalities in patients studied

still resulted in the generation of a platelet count. In these cases the paraprotein was a macroglobulin.

Examination by microscopy and the use of Isoton as diluent in the AutoCounter confirmed that the platelet-poor plasma was particle free. Red cell stroma could not be incriminated as generating the platelet count. Our work suggests that there is a reaction between the abnormal protein and the haemolysing agent. So far we have been unable to characterize this reaction but postulate that IgM molecules are aggregating, thus providing a secondary light source.

From our experience with the AutoCounter we consider that patients with monoclonal gammopathies should have platelet counts performed manually. If the count is brought on to the scale by the method of dilution (table I) considerable error is likely to be introduced.

References

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