A comparison of the erythrocyte sedimentation rate and plasma viscosity in detecting changes in plasma proteins

R. M. HUTCHINSON¹ AND R. D. EASTHAM

From the Department of Haematology, Frenchay Hospital, Bristol

SUMMARY The Westergren erythrocyte sedimentation rate (ESR) and plasma viscosity were compared in 114 patients and their correlations with total and differential plasma protein fractions were analysed.

There is a linear correlation between these two screening tests. Higher correlation coefficients were obtained between the plasma viscosity and fibrinogen and alpha and gamma globulins than with the ESR. Albumin affected the two tests in opposite directions. The ESR was falsely increased by a fall in haemoglobin even within two standard deviations from the mean.

Both tests gave an appreciable number of incorrect values—the plasma viscosity in 21 cases and the ESR in 33. The cause for these is discussed. It is concluded that the plasma viscosity is the more sensitive and reliable measure of changes in acute phase protein reactants and more useful for monitoring clinical progress.

Following tissue injury or inflammation, the commonest alteration in the concentrations of the various plasma proteins is the so-called acute phase plasma protein response. The protein fractions which increase include fibrinogen (its concentration can double within 24 hours), alpha-proteins (alpha-1 antitrypsin, alpha-1 anti-chymotrypsin, and alpha-1 acid glycoprotein), caeruloplasmin, and the second, third, and fourth components of complement. Subsequently, there is an increase in alpha-2 macroglobulin, followed by an increase in the plasma gamma globulins. Unlike these positive acute phase reactants, albumin is a negative acute phase reactant and tends to diminish in concentration. In a more chronic pathological process there may be a predominance of the negative phase response, with normal or slightly increased fibrinogen levels, normal or decreased albumin concentration, and an increase in plasma gamma globulin concentrations following antigenic exposure, beginning about 10-14 days after the initial injury (Alper, 1974).

By estimating the erythrocyte sedimentation rate (ESR) and plasma viscosity (PV) in parallel with a

series of total and differential protein fraction estimations on blood samples obtained from a group of patients, an attempt has been made to determine which of these two tests gave the most direct indication of changes in the plasma proteins. In this way, it was hoped that it might be possible to decide which of these two simple screening tests would be most useful in the assessment of severity and duration of disease known to alter the normal plasma protein pattern.

Methods and materials

Blood samples were obtained from 114 unselected patients attending a general medical and rheumatology outpatient clinic. Their clinical diagnoses were not considered during this investigation. The ESR was determined within four hours of collection by the Westergren technique (as described by the International Committee for Standardization in Hematology, 1973), and the normal range was taken as 0-5 mm for men and 0-7 mm for women, when read after one hour. Although Bull and Brecher (1974) have shown the Wintrobe method better for picking up changes in macromolecules in blood, Westergren is better for detecting changes in established disease (where there is marked increase

¹Present address and requests for reprints: Department of Haematology, Leicester Royal Infirmary

of macromolecules) (Dacie and Lewis, 1975). The coefficient of variation obtained from 10 replicate estimations on a blood sample with an ESR of 50 mm/hour was 6.4%.

The PV was estimated at 25°C by means of a capillary viscometer (Harkness, 1971) with a normal range for both sexes of 1.5-1.72 cP and a coefficient of variation for the method of 1.06%.

Full blood counts were carried out on a Coulter 'S' Counter, male patients being considered as anaemic when their haemoglobin level was less than 13 g/dl, the corresponding figure being 11.5 g/dl for females (Dacie and Lewis, 1975).

Total and differential serum protein levels were estimated by AutoAnalyzer and cellulose acetate electrophoresis (Varley, 1967). Fibrinogen concentrations were measured in EDTA plasma by a nephelometer, with a normal range of 1.5-4.0 g/l (Watson, 1961).

Results

In Table 1 regression analyses are shown comparing ESR results with the corresponding plasma viscosity, serum total globulin, and plasma fibrinogen. Because a low haematocrit caused false positive results with the ESR, further regression analyses were carried out on non-anaemic patients only. These were expanded to embrace the differential globulin concentrations and are shown in Table 2. The corresponding analyses comparing plasma viscosity results with serum protein fractions and plasma fibrinogen are shown in Table 3.

A significant inverse correlation between serum

Table 1 *Regression analyses to show linear correlations between ESR and plasma viscosity and protein fractions, without exclusion of anaemic patients*

	r	No.	SE	P
ESR and plasma viscosity	0.65	97	0.12	<0.001
ESR and total globulin	0.16	114	0.09	0.1
ESR and fibrinogen	0.25	97	0.11	<0.1 >0.05

Table 2 *Regression analyses to show linear correlations between ESR and plasma viscosity and protein fractions, after exclusion of results from anaemic patients*

	r	No.	SE	P
ESR and plasma viscosity	0.69	73	0.12	<0.001
ESR and total globulin	0.52	57	0.13	<0.001
ESR and fibrinogen	0.55	73	0.12	<0.001
ESR and gamma globulin	0.52	53	0.12	<0.001
ESR and alpha-2 globulin	0.40	53	0.14	<0.1 >0.05
ESR and total alpha globulin	0.089	53	0.14	<0.1

Table 3 *Regression analyses to show linear correlations between plasma viscosity and protein fractions, without exclusion of results from anaemic patients*

	r	No.	SE	P
Plasma viscosity and total globulin	0.76	114	0.09	<0.001
Plasma viscosity and fibrinogen	0.81	114	0.09	<0.001
Plasma viscosity and gamma globulin	0.56	114	0.09	<0.001
Plasma viscosity and total alpha globulin	0.58	114	0.09	<0.001
Plasma viscosity and alpha-2 globulin	0.51	114	0.09	<0.001

Table 4 *Correlation coefficients (r), standard error (SE), and probabilities for albumin against total globulin and fibrinogen*

	r	No.	SE	P
1 Albumin v total globulin	-0.58	114	0.09	<0.001
2 Albumin v fibrinogen	-0.343	114	0.09	<0.1 >0.05
3 Fibrinogen v globulin	0.381	114	0.09	<0.1 >0.5

albumin and globulin concentrations and a marginally significant inverse correlation between serum albumin and plasma fibrinogen concentrations were found (Table 4), with a direct correlation between plasma fibrinogen and serum globulin.

The incidence of results above or below the normal limits for each test (ESR and PV) when the serum globulin and/or plasma fibrinogen concentrations were normal or increased above normal was compared (Table 5). The PV result was raised above normal when both serum globulin and plasma fibrinogen results fell within normal limits in one blood sample, while PV results fell below the normal upper limit in the presence of increased plasma fibrinogen and/or serum globulin concentration in 20 blood samples. The ESR was increased above normal in the presence of normal concentrations of plasma fibrinogen and serum globulin in 21 samples, and was below the upper normal limits when plasma fibrinogen and/or serum globulin concentrations were abnormally increased in 12 samples.

Discussion

There is a direct correlation between ESR and PV which can be described by the equation $y = 107.08x - 162.47$, where y = ESR in mm at one hour and x = PV in centipoise at 25°C, $r = 0.69$ (Figure). Crockson and Crockson (1974), using a different method for PV estimation and a modified Westergren ESR technique, reported a similar direct correlation, with $r = 0.47$.

Table 5 Failures of the Westergren ESR and plasma viscosity to predict fibrinogen and globulin concentration

	Concentration of fibrinogen and globulin				Totals	True result	False result
	Fib. normal Glob. normal	Fib. raised Glob. normal	Fib. normal Glob. raised	Fib. raised Glob. raised			
ESR raised PV raised	0	28	0	21	49	49	0
ESR normal PV raised	1	2	1	1	5	0	5
ESR raised PV normal	21	10	2	0	33		33
ESR normal PV normal	19	3	2	3	27	19	8
Totals	41	43	5	25	114	68	46

Summary of table

Plasma viscosity gave false positive results with normal proteins in one and false negative results with raised globulin and/or fibrinogen in 20. ESR gave false positive results with normal proteins in 21, and false negative results with raised globulin and/or fibrinogen in 12.

The plasma viscosity yielded false results in 21 cases.

The ESR yielded false results in 33 cases.

Figure Relationship between ESR and plasma viscosity, derived by regression analysis of the two parameters on each sample of blood from 73 non-anaemic persons. The relationship is significant at the 5% level

As the correlations with individual protein fractions were examined, it was found that when anaemic and non-anaemic patients were considered together, the ESR gave much lower correlation values than did the PV results, and when the anaemic patients' results were excluded correlations with the

ESR results improved but were still lower than corresponding PV correlations (Tables 1, 2, and 3). The highest direct correlation was found between PV and plasma fibrinogen (Table 3). This confirms the finding of Eastham and Morgan (1965).

The ESR is a test in which various different hydrophilic protein fractions cause red cell aggregation, and the rate of red cell sedimentation depends directly on the size of aggregates formed (the rate of sedimentation being directly proportional to the square of the radius of the aggregate—Stokes' Law), and the rate at which these aggregates or pseudo-agglutinations are formed. The sedimentation of red cells in plasma is complex and consists of three phases, namely, aggregation, phase of maximum sedimentation, and packing which vary greatly and which are interrelated in a complicated manner (Eastham *et al.*, 1958). Thus changes in plasma protein pattern cause red cell aggregation, and the rate of fall of aggregates is affected by the initial haematocrit. Plasma fibrinogen, total globulin, and macroglobulins all enhance red cell aggregation and sedimentation (Scherer *et al.*, 1975). In this study, however, the ESR failed to give a highly significant correlation with the total alpha proteins. Their rise in concentration antecedes the changes in fibrinogen occurring in the acute phase reaction. Thus Harkness (personal communication, 1975) found the changes in the ESR to lag behind those in PV by 24 to 48 hours after an acute myocardial infarction.

Albumin normally disperses rouleaux and thus retards red cell aggregation and hence tends to reduce the ESR.

The PV is also a test used to detect changes in plasma protein pattern; the more intensely hydro-

philic proteins with high molecular weight, such as fibrinogen or macroglobulins, increase plasma viscosity to a greater extent weight for weight than albumin. However, an increase in any plasma protein fraction, including increase in albumin concentration, directly increases plasma viscosity (Eastham and Morgan, 1965). Thus the plasma albumin concentration affects the two tests differently. Harris (1972) was able to show that the raised ESR frequently found in some geriatric patients was directly related to reduced plasma albumin levels which, by promoting rouleaux, increases red cell aggregation in the presence of normal concentrations of fibrinogen and globulin. The plasma viscosity values in these patients fall within the normal range. Recently the importance of low plasma viscosity values below the lower normal limit as an indication of low immunoglobulin levels has been described (Basterfield, 1975) and adds to the value of this test; there is no such thing as an ESR below normal.

In the present study the effect of anaemia on the ESR as well as the effects of the protein fractions have already been mentioned (Tables 1 and 2). The normal haematocrit is different for adult males and females, and this is reflected in the differences between the normal upper limits for the ESR in the two sexes. We found that even when the haematocrit lay within 2 standard deviations below the mean, the ESR was artificially raised given the same total globulin and fibrinogen. In addition, valid results are obtained only if the ESR is performed within 4 hours of collection, and results obtained are affected by the ambient temperature at which the test is performed.

Plasma viscosity is not affected directly by changes in the haematocrit, nor is it affected by storage of plasma for up to 48 hours (Harkness, 1971).

When the ESR and PV results are compared with plasma fibrinogen and/or globulin concentrations simply defined as above or below the upper limits of normal, it was found that both tests gave 'false negative' and 'false positive' results (Table 5). The ESR gave more 'false positive' results in the presence of normal plasma protein results even when anaemic patients were excluded, and the PV gave more 'false negative' results in the presence of increased plasma fibrinogen and/or globulin value. It is probably therefore of significance that there was an inverse correlation between serum albumin values and serum total globulin concentrations. Many conditions in which raised plasma globulin levels are found are associated with falling serum albumin concentrations. Such changes in plasma protein patterns would tend to increase the ESR by removing the anti-aggregation effects of albumin, while at the same time reducing the plasma viscosity in proportion to the fall in serum albumin.

It is considered that the PV is a simple test rapidly carried out, with good precision, which is sensitive to acute phase protein reactants and is useful in the monitoring of clinical progress. The ESR is also a simple test but it has the disadvantage of a time limit for performance, and the complications caused by abnormally high or low haematocrit values with no established satisfactory method for correction for anaemia, and no way of detecting abnormal protein patterns in the presence of raised haematocrit.

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