A QUANTITATIVE STUDY OF THE ASSOCIATIONS BETWEEN PLASMA PROTEINS AND THE SEDIMENTATION RATE, PLASMA VISCOSITY AND C-REACTIVE PROTEIN

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SYNOPSIS

Multiple regression analysis of the data obtained from 122 individuals with varying ranges of erythrocyte sedimentation rate (ESR) values to determine the extent to which ESR, plasma viscosity (PV) and C-reactive protein (CRP) were related to fibrinogen (FIB), globulin (GLOB) and albumin (ALB) levels revealed that both the ESR and PV were influenced by an interplay of the 3 proteins. CRP changes on the other hand, appeared in paralled with changes in FIB and GLOB only. From the values of the coefficient of determination (R^2) obtained it is concluded that the 3 proteins have a greater influence on the ESR than on PV.

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INTRODUCTION

Reported studies on the mechanism of sedimentation of erythrocytes reveal that a complex interplay of several factors is involved in influencing the ESR (1). In general the sedimentation rate is affected by properties of the erythrocytes, plasma and by the mechanical or technical factors (2).

The measurement of PV affords a useful non-specific index of disease which some believe to be superior to that proided by the ESR; it is a particularly useful index of the severity of hyperproteinemic states and also of the response to treatment of myeloma and macroglobulinemia (3). A comparison between PV and ESR in various disease states has been reviewed by Harkness (4).

Quantitative measurement of CRP is an exquisitely sensitive and responsive indicator of the inflammatory and/or necrotic process (5,6,7,8,9). For the CRP to be an effective indicator of the inflammation and/or necrosis, it is important to obtain an accurate quantitative result at a clinical opportune time, that is when inflammation is present (10).

In this paper we present the results of a statistical analysis designed to assess the extent of any relationship between ESR, PV and CRP and the 3 proteins, FIB, GLOB and ALB.

MATERIALS AND METHODS

Our analysis is based on data from a study involving 122 individuals, consisting of 34 normal individuals and 88 inpatients with various chronic and acute diseases admitted to the General Hospital, Kuala Lumpur. The inclusion of both normal individuals and patients in the samples ensures as wide a range of ESR values as possible. The influence of the red cell concentration was minimised by exclusion of cases with a haemoglobin level less than 8g/dl or more than 16g/dl.

The ESR was determined by the classical Westergren technique, with a reading taken after one hour of sedimentation. PV was measured by a viscometer from Coulter Electronics, at 37°C using the method of Harkness (11). Fibrinogen levels were determined using the calcium-thrombin method modified from Ellis and Stransky (12). Quantitative CRP was measured by laser nephlometry according to Kindmark (13). Albumin was measured by the dye-binding method and globulin level was obtained by subtracting the ALB value from the total serum protein which was measured by biuret method (14). To examine which combination of the three plasma proteins studied best explains observed variation in PV, CRP and ESR we used the technique of multiple regression analysis (15).

RESULTS

The data base used in our analysis consists of the values of the following variables measured from the blood of 122 individuals:

Plasma Viscosity in mg/1

Erythrocyte sedimentation rate in mm/hr

Fibrinogen, globulin and albumin in g/l

Summary statistics for each of these six variables are given in (Tab I). This table indicates the ranges of the six variables for the individuals in our study.

In this paper, we are examining the extent to which each of the three dependent variables, PV, ESR and CRP, is related to the three explanatory variables, FIB, ALB and GLOB. We therefore give in (Figs. 1-9) plots of each of PV, CRP and ESR against each of FIB, GLOB and ALB.

In some of these plots, particularly (Figs. 1,2,4,7) we see that there are clear linear relationships between the variables plotted. Ther is no evidence of any non-linear relationships between any of the pairs of variables.

A preliminary examination of the data and the results of simple linear regression analyses revealed that the data for three of the 122 individuals in the study were somewhat unusual. The observations for these individuals are labelled A, B and C in some of the plots given in (Figs. 1-9). Individual A had a relatively small value of FIB (0.21), a small value of GLOB (22.0) and a large value of ALB (48.0) whilst the ESR for this individual (28.0) is not very high. Individuals B and C both have abnormally large values of FIB, namely 13.8 and 14.3 respectively. Since the data for these individuals are clearly discordant and are likely to have an undue impact on the results of the regression analysis, we have omitted the data for these three individuals from further analyses.

TABLE I: SUMMARY STATISTICS FOR THE SIX VARIABLES

Variable	Mean	S.D	Range
PV	1.43	0.16	1.10—2.00 centipoise
CRP	40.10	64.40	1.40—290.0 mg/1
ESR	46.46	44.30	1.0—150.0 mm/hr
FIB	4.29	2.35	0.21—14.30 gm/1
GLOB	37.57	5.92	27.0—51.0 gm/1
ALB	40.75	6.42	22.0—57.0 gm/1

Sample size = 122

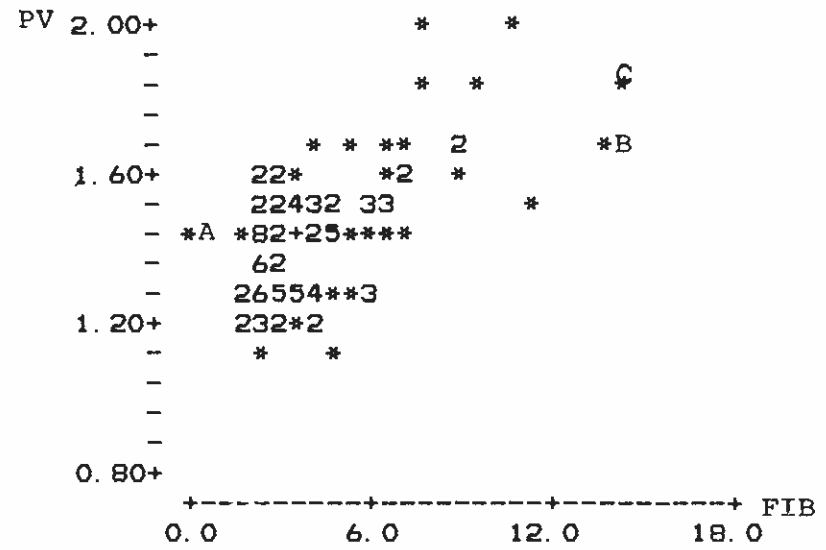

FIG. I: A plot of PV against FIB
Note: Figures in plot indicate number of coincident

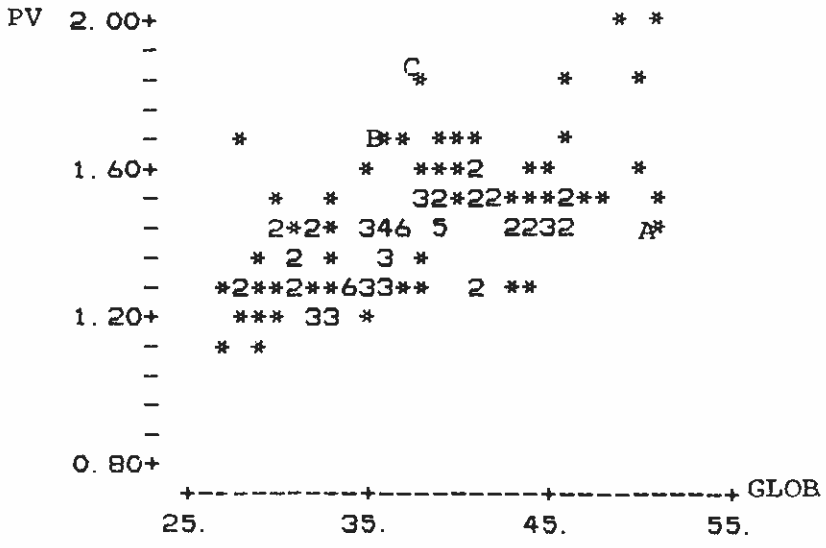

FIG. 2: A plot of PV against GLOB
Note: Figures in plot indicate number of coincident points.

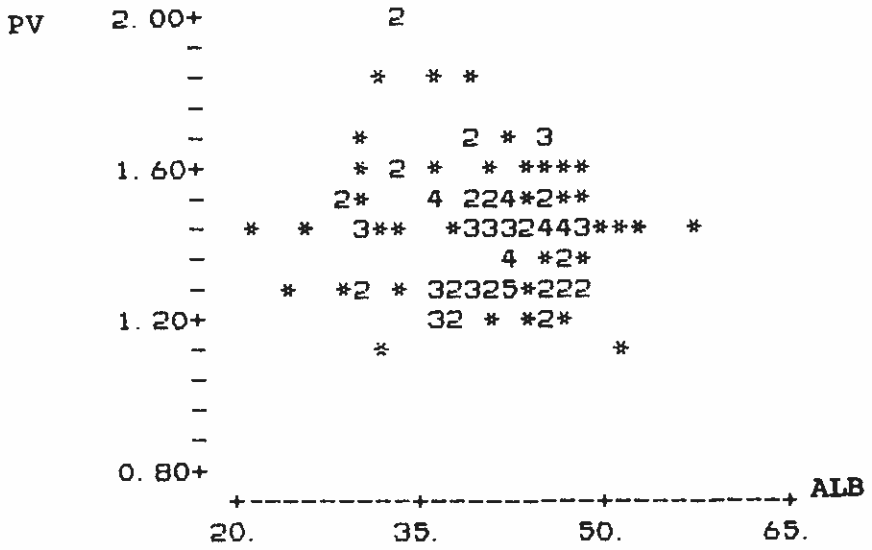

FIG. 3: A Plot of PV against ALB
Note: Figures in plot indicate number of coincident points.

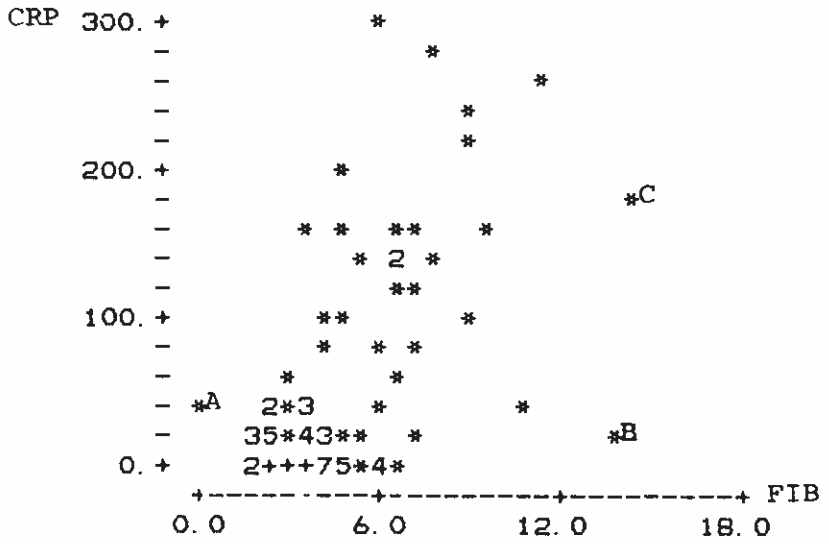

FIG. 4: A plot of CRP against FIB
Note: Figures in plot indicate number of coincident points.

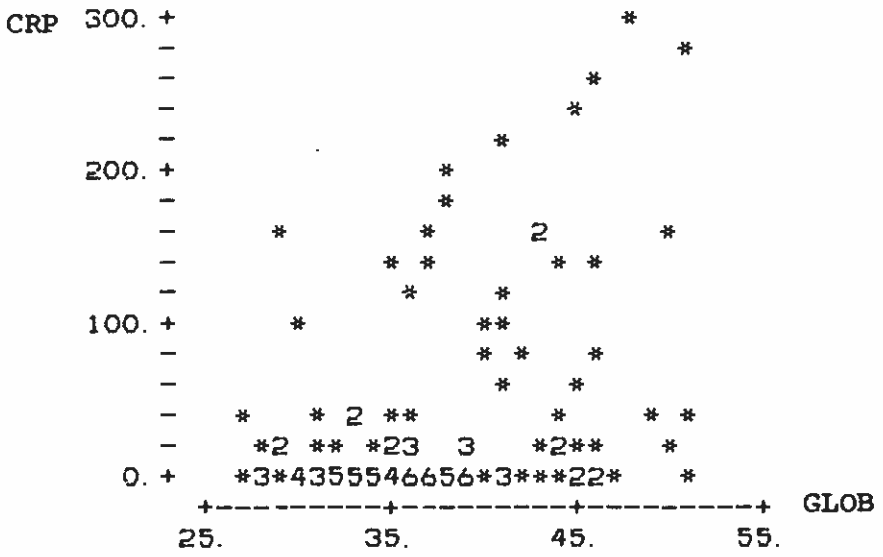

FIG. 5: A plot of CRP against GLOB
Note: Figures in plot indicate number of coincident points.

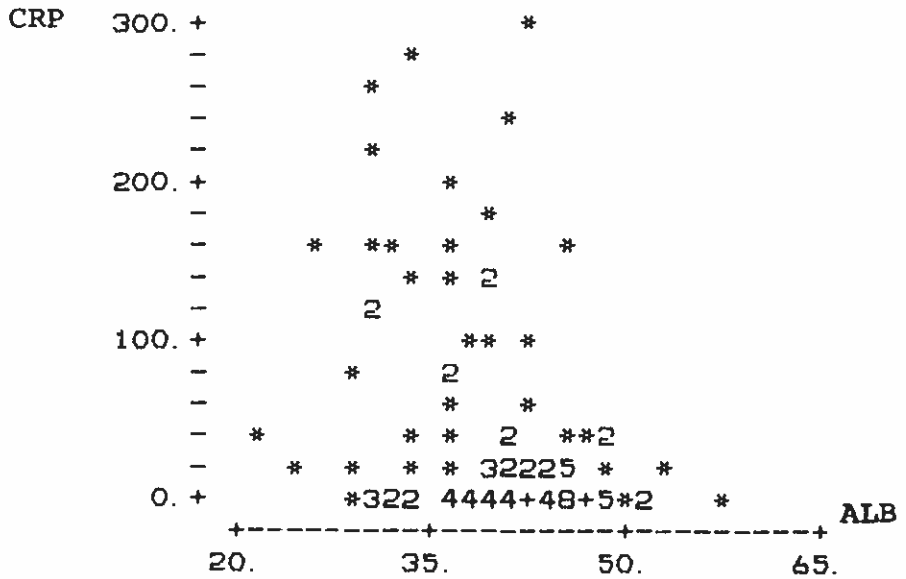

FIG. 6: A plot of CRP against ALB
Note: Figures in plot indicate number of coincident points.

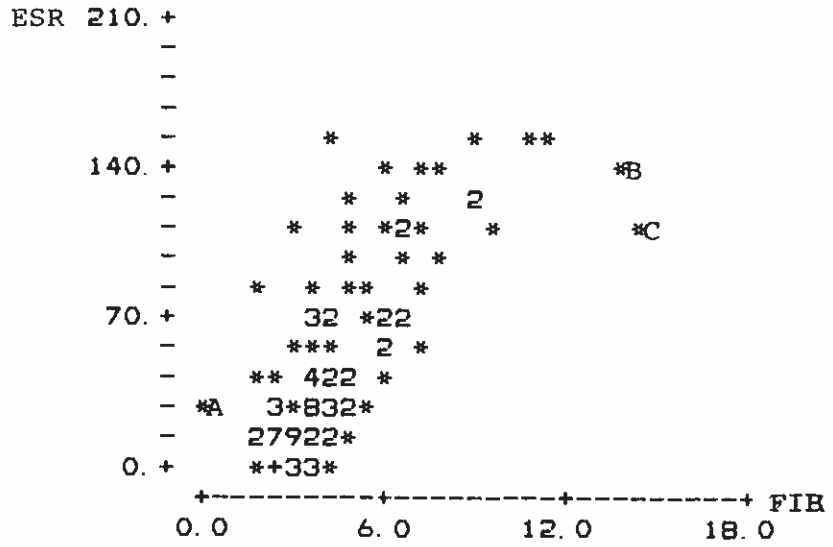

FIG. 7: A plot of ESR against FIB
Note: Figures in plot indicate number of coincident points.

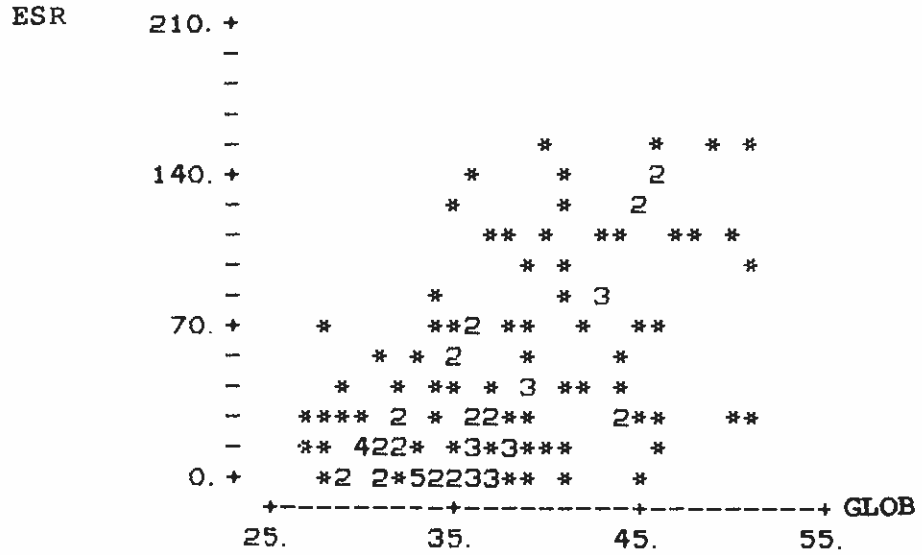

FIG. 8: A plot of ESR against GLOB
Note: Figures in plot indicate number of coincident points.

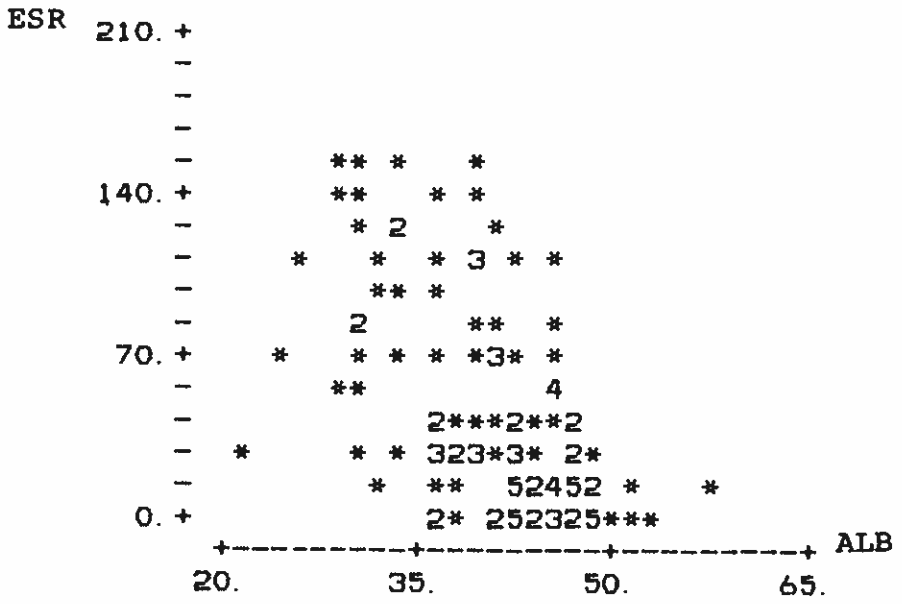

FIG. 9: A plot of ESR against ALB
Note: Figures in plot indicate number of coincident points.

Given in (Tab. II) are the results of regression analyses to study the extent of the linear relationships between the three dependent variables (PV, CRP and ESR) and the three explanatory variables (FIB, GLOB and ALB). We find that there is a significantly high positive correlation between all pairs of variable considered, apart from between PV and ALB.

Focussing specifically on the three explanatory variables, we find that there are significantly high positive correlation ($p < 0.001$) between each pair. The correlation matrix between the three pairs is given in table III.

Thus ALB is negatively correlated with both FIB and GLOB which are themselves positively correlated.

The results of a multiple regression analysis suggest that both PV and ESR are jointly related to all the three proteins. Regression models with just one or two of the three proteins are not as satisfactory in describing observed variability in either PV or ESR. However the variation in the values of CRP is best explained by just the two proteins FIB and GLOB. Although the extra variation explained by GLOB, after allowing for FIB, is not quite significant at the 5% level ($p = 0.056$) it is sufficiently large to warrant including both proteins in the regression model.

Details of the fitted regression equations are given below. The standard error of the regression coefficients in each equation is given in parentheses.

TABLE II: RESULTS OF SIMPLE LINEAR REGRESSION ANALYSES

Regression Equation	Correlation Coefficient	n	p-values
PV = 1.226 + 0.047 FIB	0.58	119	< 0.001
PV = 0.798 + 0.017 GLOB	0.612	119	< 0.001
PV = 1.536 − 0.0028 ALB	−0.110	119	> 0.10
CRP = −51.10 + 21.667 FIB	0.669	119	< 0.001
CRP = −116.91 + 4.163 GLOB	0.382	119	< 0.001
CRP = 198.86 − 3.904 ALB	−0.383	119	< 0.001
ESR = −27.48 + 17.48 FIB	0.790	119	< 0.001
ESR = −111.60 + 4.187 GLOB	0.562	119	< 0.001
ESR = 214.97 − 4.147 ALB	−0.595	119	< 0.001

TABLE III: CORRELATION MATRIX BETWEEN ALBUMIN GLOBULIN AND FIBRINOGEN

FIB			
GLOB	0.28	1	
ALB	− 0.37	− 0.38	1
	FIB	GLOB	ALB

$$PV = 0.3627 + \underset{(0.0057)}{0.0438}\ FIB + \underset{(0.0018)}{0.0140}\ GLOB + \underset{(0.0018)}{0.0086}\ ALB$$

(R^2 = 59.30%; n = 119)

$$ESR = -18.06 + \underset{(1.27)}{12.85}\ FIB + \underset{(0.04)}{1.92}\ GLOB - \underset{(0.39)}{1.52}\ ALB$$

(R^2 = 73.35%; n = 119)

$$CRP = -100.9 + \underset{(2.39)}{19.88}\ FIB + \underset{(0.80)}{1.53}\ GLOB$$

(R^2 = 46.50%; n = 119)

The coefficient of determinations, R^2, is the square of the correlation between the observed values of a dependent variable and the corresponding predicted values under an assumed regression model. It can be interpreted as the proportion of variation in the dependent variable explained by the independent variable. Here, R^2, is the amount of variability in the values of ESR, PV or CRP accounted for by the proteins FIB, GLOB and ALB. We see that this is highest for ESR, showing that ESR is more strongly related to the proteins than PV or CRP.

DISCUSSION

The most influential factors in determining the sedimentation rate were found by Meyers *et al* (1953) to be plasma/cell ratio, fibrinogen, alpha-2 globulin and gamma globulin (1). These authors also noted the negative influence of albumin on the ESR.

Our results are in agreement with the above findings in that the ESR is related to all the three proteins, FIB, GLOB and ALB. This suggests that there exists a direct cause and effect relationship between the levels of these three proteins and the ESR. Increase in FIB and GLOB levels in the plasma will decrease the zeta potential of the erythrocyte which will undergo increased rouleaux formation thus increasing the ESR value. On the other hand ALB acts in the opposite direction. Therefore an increase in the FIB and GLOB and a decrease in ALB will result in an ESR value above that of normal.

Plasma viscosity which is a more useful parameter to measure in diseases involving changes in the plasma itself is also found to be influenced by all three proteins mentioned. The concept of viscosity is simply the resistance offered by a liquid to attempts to change its shape (3). Since all the three proteins investigated are part of the content of the plasma, changes in their levels are expected to result in a change in the viscosity of the plasma.

Changes in the CRP on the other hand are not found to be related to all the three proteins but to only two proteins FIB and GLOB. Being an acute-phase protein that can be directly measured, the CRP value is not influenced by the levels of the three proteins as in the case of ESR and PV. The CRP is more directly related to the condition of the disease investigated. The multiple regression analysis in this case is more of a comparison between the levels of these acute-phase proteins rather than information on the extent of any influence exerted by the three proteins FIB, GLOB and ALB over the CRP level.

From the values of the R^2 obtained, we conclude that the three proteins have a greater influence on the sedimentation rate of erythrocytes than on the viscosity of plasma. Changes in CRP levels, on the other hand, appear to occur in parallel to changes in FIB and GLOB alone. This reflects the fact that the three proteins, CRP, FIB and GLOB, appear in circulation together under the influence of various clinical stresses.

It is not our purpose to compare the relative usefulness of the three parameters in predicting disease activity. The merits and demerits of each parameter have been discussed previously (16), but in general the ESR, while being simple to measure, and generally popular with clinicians, may be quite insensitive as an indicator of disease activity (e.g. infection) when the patient has an underlying disease that results in changes in the ALB level, as in protein-losing nephropathy, enteropathy or liver disease. The more useful role of CRP in these situations has been documented earlier (17). When the disease activity has a direct effect on the plasma, protein levels, in particular in para-proteinemias, the PV measurement is superior to the ESR measurement in the assessment of disease activity. Clinicians must be prepared to recognise the limitations of these parameters in defined clinical settings, and to utilise the relevant measurement in some special circumstances, rather than to rely solely on one parameter for all clinical settings.

ACKNOWLEDGEMENTS

We are grateful to the staff to the Coagulation Laboratory, Haematology Division and the Research Laboratory, Biochemistry Division, Institute for Medical Research, Kuala Lumpur, Malaysia for their technical assistance.

REFERENCES

1. Meyers AJ, Trevorrow V, Washburn AH, Mugrage ER: Quantitative studies of the influence of plasma proteins and hematocrit on the erythrocyte sedimentation rate. Blood 1953; 8: 893.
2. Miale J. The Blood. In: Laboratory medicine: Hematology. 3rd ed New York, The C.V. Mosby Company. 1981: 325.
3. Phillips MJ: Plasma and whole blood viscosity. Br J Haematol 1976; 34: 347.
4. Harkness J: The viscosity of human blood plasma; its measurement in health and disease. Biorheology 1971; 8: 17.
5. Anderson HC, McCarty M: Determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever. Am J Med 1950; 8: 445-55.
6. Fischer CL, Gill CW. Acute phase proteins, serum protein abnormalities: Diagnosis and clinical aspects. Boston, Little, Brown and Co. 1971: 331-50.
7. Fischer CL, Gill CW, Forrester MG, et al: Quantitation of acute phase proteins post-operatively. Am J Pathol 1976; 66: 840-6.
8. Hoyashi H, Lo Crippo GA: C-reactive protein-potential significance of quantitation in patients with chronic disease. Henry Ford Hospital Med J 1972; 20: 91-6.
9. Werner M; Serum protein changes during the acute phase. Clin Acta 1969; 25: 299-305.
10. Gill CW, Bush WS, Burleigh WM, Fischer CL: An evaluation of a C-reactive protein assay using a rate immunonephlometric procedure. Am J Clin Pathol 1981; 75: 50-5.
11. Harkness J: New Instrument for the measurement of plasma viscosity. Lancet 1963; I: 2: 280-1.

12. Ellis RC, Stransky A: A quick and accurate method for the determination of fibrinogen in plasma. J Lab Clin Med 1961; 58: 477.

13. Kindmark CP: Concentration of C-reactive protein in sera of healthy individuals. Scand J Clin Lab Invest 1972; 29: 407.

14. Keyser JW: Rapid estimation of albumin and total protein in small amount of blood serum. Clin Chim Acta 1961; 6: 445.

15. Draper NR, Smith H. Applied Regression Analysis. 2nd ed. Yew York, Wiley. 1981.

16. Roshidah I, Khalid H: The Influence of fibrinogen and other plasma proteins on the ESR and plasma viscosity. Med J Malaysia 1985; 40 (3): 191-5.

17. Sliwinski AJ, Weber LD, Nashel DJ: C-reactive proteins vs Erythrocyte sedimentation rate: A comparison of effectiveness as an infection marker in patients undergoing peritoneal dialysis. Arch Pathol Lab Med 1983; 107: 387-8.