Viscosity of plasma in patients with rheumatoid arthritis

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SUMMARY The viscosity of plasma (PV) was investigated in 27 outpatients with classical or definite rheumatoid arthritis (RA) according to the American Rheumatism Association (ARA) criteria. The measurements showed a significantly raised PV in patients with RA ($p < 10^{-5}$) compared with a control group. There was a positive correlation between the PV and the blood erythrocyte sedimentation rate (B-ESR), and the increase in the PV is largely explained by increased concentrations of the plasma proteins, fibrinogen, and IgG.

Key words: B-ESR, fibrinogen, IgG, blood sedimentation.

It is well established that the PV and the B-ESR increase in RA,¹⁻³ and it has been shown that the PV is affected mainly by the plasma proteins, fibrinogen, and gammaglobulin.⁴⁻⁶

We have investigated the relationship between PV, B-ESR, and the concentrations of plasma proteins likely to affect viscosity both in patients with RA and healthy subjects.

Patients and methods

PATIENTS

Twenty seven outpatients, 17 female and 10 male, mean age 63 (SD 10) years, with classical or definite RA according to the ARA criteria were investigated. All were receiving aurothiomalate or penicillamine and/or non-steroidal anti-inflammatory drugs; none had received corticosteroids during the previous six months. Twenty five healthy subjects, 15 female and 10 male (aged 39 (10) years), were used as a control group.

Blood specimens were taken by venepuncture in the morning after eight hours of fasting. Ethylenediaminetetra-acetate (K_2EDTA 4.0 mmol/l blood) was used as anticoagulant, and plasma was separated from the blood cells by centrifugation within one hour of venepuncture.

MEASUREMENTS

The erythrocyte sedimentation rate (B-ESR) was

measured by the methods of Westergren (1926) and performed within one hour of venepuncture.

The viscosity of the plasma (PV) was measured using a Wells-Brookfield microviscometer, model LVT cone/plate⁷ at 37.0°C and a shear rate of 230

Fig. 1 Viscosity of plasma measured at 37 °C. Twenty five healthy subjects (15 female, 10 male) and 27 patients with rheumatoid arthritis (17 female, 10 male).

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s^{-1} . The volume of the specimen in the cup was 1.1 ml.⁸ Measurements were performed within four hours of venepuncture. All results are mean values from duplicate measurements.

Plasma concentrations were determined as follows: (a) albumin (P-albumin) by an endpoint

turbidometric assay using antibodies against human albumin; (b) fibrinogen (P-fibrinogen) by a kinetic turbidometric assay using antibodies against human fibrinogen; (c) fibronectin (P-fibronectin) by a nephelometric assay using antibodies against fibronectin; (d) immunoglobulins (P-IgG, P-IgA, P-IgM) by a nephelometric assay using antibodies against human immunoglobulins.

Fig. 2 Correlation between four different quantities (a, b, c, d) measured in blood or plasma and the viscosity of plasma measured at 37 °C. $\square$ = healthy controls; $\blacktriangle$ =patients with rheumatoid arthritis; r is the coefficient of correlation.

Table 1 Comparison of quantities measured in blood, plasma, or serum from 25 healthy subjects and 27 patients with rheumatoid arthritis*

System† and component	Unit	Controls (n=25)	Patients with RA (n=27)	U test (p value)
P-viscosity	mPa.s	1.38 (0.09)	1.55 (0.12)	<10 ⁻⁵
B-ESR	mm/l h	5.0 (3.2)	27.4 (13.5)	<10 ⁻⁵
P-albumin	μmol/l	581 (40)	501 (51)	<10 ⁻⁵
P-fibrinogen	μmol/l	11.2 (2.4)	15.6 (3.1)	<10 ⁻⁵
P-IgG	g/l	9.9 (2.2)	13.2 (5.1)	<10 ⁻⁵
P-IgA	g/l	1.8 (0.6)	3.1 (1.6)	<10 ⁻⁵
P-IgM	g/l	1.5 (0.7)	1.4 (1.0)	NS
P-plasminogen	μmol/l	1.6 (0.3)	1.8 (0.3)	NS
P-fibronectin	μmol/l	1.1 (0.4)	1.2 (0.2)	NS
S-ferritin	μg/l	47 (53)	42 (47)	NS
P-antithrombin III	Arb. unit	1.1 (0.2)	1.2 (0.2)	NS

*Levels are given as mean (standard deviation) and are tested for differences by the Mann-Whitney U test. Two tailed probabilities are shown.

†B=blood; P=plasma; S=serum.

IgM), and plasminogen (P-plasminogen) by quantitative immunoelectrophoresis; and (e) antithrombin III (P-antithrombin III) by Coatest (Kabi, USA).

Ferritin (S-ferritin) was determined by the radioimmunosorbent assay, Prist (Pharmacia AB, Sweden).

ANALYSIS

Stepwise multiple linear regression analysis⁹ was performed on data from the combined groups of individuals (n=52). The dependent variable, PV, was expressed as a linear function of the independent variables, P-albumin, P-fibrinogen, P-IgG, P-IgA, and P-IgM. The protein concentrations were all greater than 1 g/l.

Results

The results of the PV measurements are shown in Fig. 1. PV is independent of sex in both groups. In Table 1 the means and standard deviation of the measured quantities are given for controls and patients with RA. PV, B-ESR, P-fibrinogen, P-IgG, and P-IgA were higher in patients with RA than in the controls ($p<0.001$). PV is presented graphically against each of the above parameters (Figs 2a–2d). The corresponding simple correlations were all significant. P-albumin was lower in the patients with RA than in the controls. The influence of the individual plasma protein concentrations on PV was found by stepwise multiple linear regression analysis. Of the five different protein concentrations investigated simultaneously, P-fibrinogen was the best predictor of PV, followed by P-IgG and P-IgM. These three variables all contributed independently of each other to the PV variation ($p<0.05$ by *t* test),

whereas P-albumin and P-IgA did not. Fifty seven per cent of the total variation of PV was explained by P-fibrinogen alone. The fraction increased to 69% and further to 72% by the stepwise inclusion of P-IgG and P-IgM in the analysis. P-albumin and P-IgA were not included. The residual unexplained 28% of total variation of PV corresponds to 0.07 mPa.s when expressed as the standard error of estimate. The analytical error (repeatability) of the PV results was estimated by replicate measurements to 0.045 mPa.s (SD).

Discussion

Our results are in agreement with earlier investigations in finding a significant rise in PV and a correlation between PV and B-ESR.⁴ PV appeared to be a more sensitive test than B-ESR as the PV was abnormal earlier in the disease,¹⁰ but we find that the patients with RA are better distinguished from the controls by B-ESR than by PV (Fig. 2a). One explanation might be the greater experimental error shown by a cone/plate viscometer in our measuring conditions: 3% (coefficient of variation) compared with 0.5% by the capillary viscometer.^{10 11} Higher relative values of B-ESR and PV for the patients with RA than for the controls¹⁰ may also lead to a different degree of separation. PV in the combined group of controls and patients with RA is mainly determined by P-fibrinogen. The second most important protein concentration is P-IgG. The effect of P-IgM is just measurable. The much lower P-albumin and the higher P-IgA in patients with RA did not affect PV. P-plasminogen, P-fibronectin, S-ferritin, and P-antithrombin III were unchanged in RA. We do not expect these

proteins to influence PV because of their low mass concentrations, less than 1 g/l. The diagnostic value of P-fibrinogen, P-IgG, and P-IgA in RA seems comparable with that of PV or B-ESR.

Smoking, defined as smoking at least five cigarettes a day in the previous three months, is known to increase viscosity.¹² In our control group 13 persons were smokers and eight non-smokers, but we found no significant difference in PV between these two groups. On the other hand, P-antithrombin III was significantly higher in smokers than in non-smokers. This observation deserves further investigation.

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