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Haemorheological variables as a rheumatoid arthritis activity indicator

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Abstract. *Objective*: To investigate if blood hyperviscosity in RA patients is due to a reduced erythrocyte deformability and, therefore, turning it into a reliable activity indicator, as well as a therapy follow-up marker for this pathology.

Methods: (1) The haemorheological profile consisting of erythrocyte deformability, blood and plasma viscosity, and erythrocyte membrane fluidity was determined in 24 AR patients and 17 healthy controls. (2) A 4 year follow-up was carried on in 16 patients monitoring blood viscosity, erythrocyte deformability and biochemical variables in relation to clinical assessment of disease activity (Disease Activity Score "DAS 28-4").

Results: Erythrocyte deformability and membrane fluidity were impaired in RA patients compared to controls ($p < 0.001$). Blood viscosity was significantly increased and correlated with the cell rigidity index ($r = 0.85$, $p < 0.0000$) in RA patients. The follow-up showed a good correlation between haemorheological parameters and DAS 28-4 during disease evolution.

Conclusion: our results support the hypothesis that in RA, blood hyperviscosity is determined by deformability loss, which in turn is due to a membrane rigidization. This could evidenced that a widespread cell membrane damage is expressed through an impaired erythrocyte deformability, turning haemorheological parameters into reliable tools to study disease evolution. The follow-up study enabled us to confirm that erythrocyte deformability is an efficient indicator of rheumatoid arthritis activity.

Keywords: Rheumatoid arthritis, blood viscosity, erythrocyte deformability, haemorheological variables, follow-up study

1. Introduction

Since the 1940's, it is known that blood viscosity (η_b) is increased in the inflammatory syndrome, e.g., rheumatoid arthritis (RA), due to a remarkable serological immune response. Increase in plasma protein produces an increase in plasma viscosity (η_p) and erythrocyte aggregation, both leading to the hyperviscosity syndrome [1–3]. Nevertheless, attempts to relate rheological parameters modifications with disease evolution have been scarce [4,5].

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Evaluation of disease activity is cumbersome, requiring often the assessment of multiple clinical and laboratory variables, so far no single laboratory test is entirely satisfactory. Unspecific markers of inflammation, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are nonreliable either, and must be associated with clinical signs in order to evaluate disease activity [6–18]. Nevertheless, changes in these variables are included in the American College of Rheumatology Guidelines to ascertain clinical remission [17,19].

Previous studies reporting haemorheological abnormalities in rheumatic disease [21–31], led us to investigate the potential use of blood rheological parameters as indicators of disease activity. One of the haemorheological abnormalities reported in literature was diminished cell deformability [4,32].

Ernst et al. [4] reported hypochromic anaemia in RA patients, as well as a decrease in erythrocyte filterability. Hypochromic anaemia – pathognomonic in RA – produces a cytoplasmic viscosity decrease, that enhances cell deformability [33]; consequently, the observed decrease in erythrocyte filterability could be explained by cell membrane abnormality.

Recently Banerjee et al. [34] emphasised the diagnostic relevance of red cell rigidity in diseases like hypertension, diabetes mellitus and myocardial infarction. On this basis, we attempt (1) to demonstrate that in RA, the decreased erythrocyte deformability is a determinant factor of blood hyperviscosity and (2) it is a reliable disease activity indicator, useful as a follow-up marker in disease evolution during treatment.

2. Methods

2.1. Patients

Twenty-four female RA patients (mean age 48 $\pm$ 0.17 yr, ranging from 35 to 61 yr) attending an outpatient service at the Departamento de Reumatologia, Universidad Nacional de Rosario, Argentina, were included in the present study.

RA was diagnosed according to the American College of Rheumatology criteria (formerly, the American Rheumatism Association) [19,20]. Patients with cardiovascular disease, cancer, chronic infection, diabetes mellitus and heavy smoke habit (>20 cigarettes) were excluded.

Sixteen patients accepted to be included in a 4-year follow-up study. Their clinic disease activity was evaluated by means of the Disease Activity Score (DAS 28-4) [35,36], through the following equation:

$$\text{DAS 28-4} = 0.56\sqrt{\text{t28}} + 0.28\sqrt{\text{sw28}} + 0.70\ln(ESR) + 0.014GH,$$

where:

$\sqrt{\text{t28}}$ = square root of number of painful joints from 28 joints,
$\sqrt{\text{sw28}}$ = square root of number of swollen joints from 28 joints,
$\ln(ESR)$ = natural logarithm of erythrocyte sedimentation rate in mm/first hour,
GH = general health or patient's global assessment of disease activity on a 100 Visual Analogue Scale (VAS).

This equation comprises the following cut-off values: high disease activity > 5.1, low disease activity < 3.2, remission < 2.6.

At inclusion, all the patients received non-steroid anti-inflammatory drugs (NSAIDs). During the 4 yr follow-up the patients received more than one disease-modifying anti-rheumatic drug (DMRAD).

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The control group consisted of 17 female non-smoker healthy volunteers, age-matched (mean: 43 $\pm$ 0.22 yr, ranging from 31 to 62 yr).

2.2. Haemorheological assays

Blood was collected by venipuncture, with EDTA as anticoagulant (1 mg/ml). Haematocrit was assessed by microhaematocrit method.

Whole blood viscosity: measurements were performed in a Wells-Brookfield cone-in-plate viscometer at 37°C and 230 s^{-1} shear rate. Since haematocrit did not vary significantly, it was not necessary to standardise blood viscosity values measured at native haematocrit.

Plasma viscosity was measured with the same viscometer at the same shear rate already described for whole blood.

Relative blood viscosity (η_r) was calculated as follows:

$$\eta_r = \frac{\eta_b}{\eta_p},$$

where: η_b = blood viscosity; η_p = plasma viscosity.

2.3. Biochemical assays

Were assayed in serum aliquots frozen at -80°C.

Total protein was measured by colorimetric method [37], serum immunoglobulin by single radial immunodiffusion technique, and plasma fibrinogen by gravimetric method and CRP by Singer and Plotz's technique [38].

ESR was measured according to Westergreen.

RF was determined by turbidimetry [39].

2.4. Cellular assays

Erythrocyte filtration was performed in a computerised instrument [40] after Reid et al. technique [41]. A 10% suspension of washed erythrocytes was passed through a polycarbonate filter, 5 μm pore size (Nucleopore Corr., USA), using a negative filtration pressure of 10 cmH_2O. The flow time for 1 ml of RBC suspension passing through the filter was measured. Results were expressed as rigidity index (*RI*) that is an estimation of erythrocyte rigidity (inverse of erythrocyte deformability) [42]:

$$RI = \frac{T_b - T_s}{T_s} \times 100/\text{Htc},$$

where:

T_b = time of cell suspension passage through the filter,
T_s = time of PBS passage,
Htc = haematocrit (10%).

The viscosity and erythrocyte deformability by measurement an in accordance to the International Comitee for Standardization in Hematology [43].

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Erythrocyte membrane fluidity: rigidity of lipid bilayer was assessed by electron paramagnetic resonance (EPR), using the spin label 5-doxyl-stearic acid (Sigma Chemical Co., St. Louis, MO, USA), in a Bruker ER-200 spectrometer operating at X band (9800 MHz) using a flat quartz cell. The parallel component of the nitrogen hyperfine tensor ($T_{//}$) was taken as a representative parameter of lipid bilayer rigidity [44].

Erythrocyte shape: was assessed by light microscopy.

2.5. Haematological indexes

Erythrocyte count was assessed by a haemocytometer and haemoglobin by the cyanmetahaemoglobin method. From these values, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated.

2.6. Statistical analysis

Values in RA patients and their controls at inclusion time, are presented as mean ± standard deviation. Comparisons were performed by Student's t test for unpaired data. Pearson product-moment correlation coefficient was used for IR and blood viscosity, IgG, IgM, fibrinogen and $T_{//}$, and plasma viscosity with IgG, IgM and fibrinogen.

In the follow-up study, association of laboratory variables with DAS28-4 were performed using the rank correlation coefficient (Spearman's test). Significance was accepted at $p < 0.05$.

The study protocol was approved by the Ethics Committee of the Facultad de Ciencias Medicas, Universidad Nacional de Rosario, all participants signed informed consent according to the recommendations of the Declaration of Helsinki.

3. Results

In Table 1 are presented the baseline values of the variables under study. Differences between RA patients and their controls were significant ($p < 0.05$) for the biochemical variables included in the criteria for RA diagnosis (ESR, FR), for the inflammatory immunoglobulins IgG and IgM, for the pathognomonic hypochromic anaemia expression (MCHbC) and also for rheological (η_b, η_p, η_r) and cellular parameters (*RI*, $T_{//}$). Likewise, η_p was significantly correlated ($p < 0.001$) with IgG ($r = 0.61$), IgM ($r = 0.85$) and fibrinogen ($r = 0.98$), pointing out its dependence on these protein concentrations at the moment of inclusion. On the other hand, η_b yielded a highly significant correlation with *RI* ($r = 0.85$, $p < 0.0000$). In order to remove plasma influence on blood hyperviscosity, blood viscosity value (η_r) was corrected. Correlation between η_r and *RI* ($r = 0.85$, $p < 0.0000$) confirmed the role of the impaired erythrocyte deformability in RA patients hyperviscosity.

RI showed its dependence on membrane fluidity $T_{//}$ ($r = 0.546$, $p < 0.03$), while there was no correlation between *RI* and plasma proteins, namely, IgG ($r = 0.32$, $p > 0.05$) and IgM ($r = 0.33$, $p > 0.05$), and fibrinogen ($r = 0.12$, $p > 0.05$), which might be adsorbed on cell surface modifying the membrane rheology.

Evolution of disease activity during the follow-up study is depicted in Fig. 1. The corresponding values of DAS 28-4 (median and CI) are as follows: baseline, 6.98 (6.57–7.19); yr 1, 7.26 (6.9–8.24); yr 2, 5.96 (5.20–6.41); yr 3, 2.75 (2.57–3.09) and yr 4, 2.40 (1.99–2.65). Table 2 shows the association between biochemical and rheological variables with DAS 28-4 on 16 patients during the 4-yr follow-up.

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Table 1
Haemorheological and biochemical variables in RA patients and their controls at inclusion

	Patients* ($n = 24$)	Controls ($n = 17$)	Significance
ESR (mm/hr)	49.14 ± 17.91	14.00 ± 6.50	$p < 0.001$
RF (mUI)	75.51 ± 11.36	14.66 ± 2.06	$p < 0.0000$
Total protein (g/dl)	7.65 ± 0.38	6.89 ± 0.41	$p < 0.01$
Albumin (g/dl)	3.84 ± 0.44	3.77 ± 0.51	NS
Immunoglobulins (g/dl)	2.87 ± 0.25	2.56 ± 0.22	$p < 0.01$
IgG (mg/dl)	1580 ± 219	1296 ± 158	$p < 0.001$
IgM (mg/dl)	233 ± 28	186 ± 23	$p < 0.001$
Fibrinogen (mg/dl)	382 ± 80	299 ± 47	$p < 0.01$
η_p 230 (mPa.s)	1.595 ± 0.190	1.475 ± 0.064	$p < 0.05$
η_b 230 (mPa.s)	5.75 ± 1.08	4.28 ± 0.23	$p < 0.001$
$\eta_r(\eta_b/\eta_p)$	3.63 ± 0.67	2.91 ± 0.06	$p < 0.001$
Haematocrit (%)	38.45 ± 3.53	39.6 ± 2.19	NS
RI	14.79 ± 4.71	6.92 ± 1.31	$p < 0.001$
MCV (μm^3)	89.7 ± 1.6	93.15 ± 7.7	NS
$T_{//}$ (Gauss)	29.13 ± 0.10	28.82 ± 0.20	$p < 0.001$

* Data presented as mean $\pm$ standard deviation. Comparisons performed by Student's t test. Name of variables in the text.

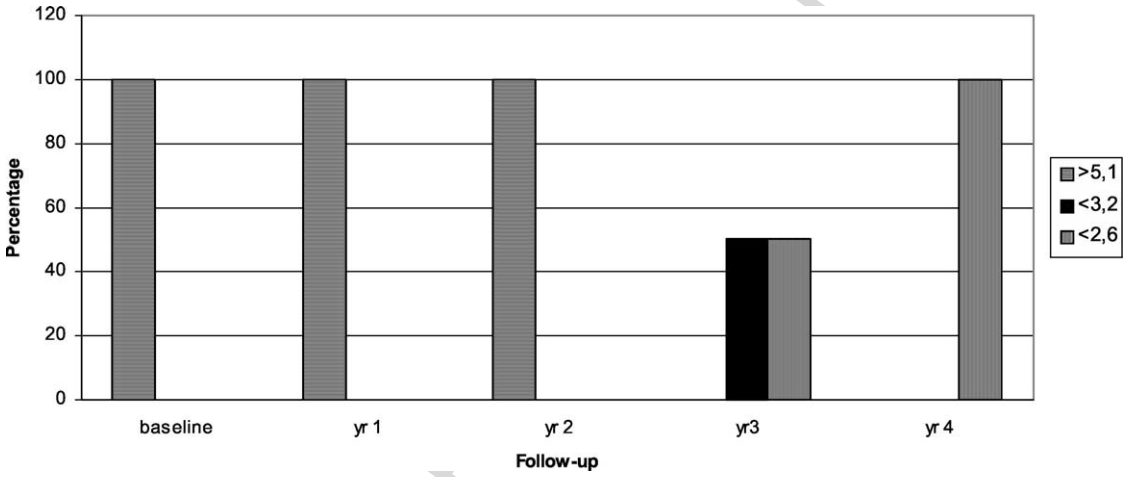

Fig. 1. Rheumatoid arthritis evolution assessed by DAS 28-4 during the follow-up study.

Table 2
Association between biochemical and haemorheological variables with DAS 28-4 in the follow-up study ($n = 16$)

	Inclusion	1 yr	2 yr	3 yr	4 yr
CRP	0.70^{*}	0.88^{*}	0.80^{*}	0.83^{**}	0.90^{**}
RF	0.25	0.15	0.20	0.09	0.12
η_p	0.30	0.35	0.25	0.12	0.30
η_r (η_b/η_p)	0.85^{*}	0.98^{*}	0.97^{**}	0.93^{**}	0.97^{**}
RI	0.80^{*}	0.99^{**}	0.95^{**}	0.81^{***}	0.99^{**}

Biochemical variables: RF, rheumatoid factor; C-RP, C reactive protein.
Haemorheological variables: η_p, plasma viscosity; η_r (η_b/η_p), relative blood viscosity; *RI*: rigidity index.
Rank correlation coefficient (Spearman's test) r_s. Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

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4. Discussion

A large number of reports on haemorheological abnormalities in RA [14–25] support the classic concept that disproteinemia – characteristic in these patients – increases plasma viscosity and consequently blood viscosity. Nevertheless, the association of haemorheological abnormalities with widespread cell modification produced by the disease have not been attempted.

Previous reports dealing with erythrocyte deformability in RA patients [4,25] are controversial, which could be due to differences in methodology or heterogeneity in patient conditions among others. Our hypothesis is that RA could modify the RBC membrane similarly as the alterations produced in the membrane of connective tissue cells (e.g., synoviocytes), therefore, turning the erythrocyte into a reliable marker of the patient clinical stage.

The haemorheological study of RA patients showed not only an increase in plasma and blood viscosity, but an increase in relative blood viscosity, which points out the involvement of a cellular factor in blood hyperviscosity. A highly significant correlation was found between relative blood viscosity and erythrocyte rigidity index ($r = 0.85$, $p < 0.0000$) supporting the hypothesis,

Erythrocyte properties were analysed to clear up the cause of its altered deformability. Haematological values showed that MCV and discocytic shape were preserved in RA patients. The slight decrease in MCHC, pathognomonic of RA [44,45], involves a lower cytoplasmic viscosity that could increase erythrocyte deformability. Thus, only a cell membrane abnormality could justify the observed decrease in erythrocyte filterability. This assumption was confirmed by means of the membrane fluidity parameter $T_{//}$, which was higher in RA patients and significantly correlated with *RI*, pointing out a loss in membrane fluidity as a cause of impaired erythrocyte deformability.

These results allow us to infer that erythrocyte deformability impairment could be an expression of a widespread cell membrane damage in RA.

Therefore, *RI* could be a more reliable RA activity indicator than some biochemical tests widely used, such as RF and η_{p}. The latter hypothesis was confirmed through a four-year follow-up on 16 patients, all of them in active clinical stage, diagnosed according to the DAS 28-4 score of the American Rheumatism Association criteria. Strikingly, neither η_{p} nor RF showed a significant correlation with DAS 28-4, whereas CRP did show a significant one. Relative blood viscosity and erythrocyte rigidity index showed a significant correlation with DAS 28-4 at inclusion and year 1, improving through year 2, 3 and 4.

Finally, as shown in Table 2, there is a strong correlation between clinical improvement with η_{r} and *RI*, and DAS 28-4 values. On the other hand, coincidentally with RA remission (DAS28-4, $<$2.6), at year 4 the haemorheological parameters attained normal values: $\eta_{\mathrm{r}} = 2.74$ (2.21–3.30); $RI = 8.00$ (5.00–10.77).

In conclusion, these results support the hypothesis that in RA a widespread cell membrane damage is also expressed in the erythrocytes, and might be detected through the alterations of their rheological properties, thus rendering these cells as reliable tools for evaluating activity along the therapeutic intervention.

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