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Erythrocyte aggregation in rheumatoid arthritis: Cell and plasma factor's role

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Abstract. Increase in erythrocyte aggregation (EA) is pathognomonic for rheumatoid arthritis (RA), and its estimation through erythrocyte sedimentation rate (ESR) is part of DAS 28-4 activity diagnosis, with low correlation with EA and that does not discriminate the contribution of cell factors that increase aggregation.

Objective: To analyse cell and plasma factors that might be involved in EA increase, to understand how RA affects blood components, thus modifying blood fluid behavior.

Methodology: One hundred women presenting active RA were compared with age-matched controls (C). EA was measured by transmitted light, obtaining two parameters: $2k_2n_0$, characterizing the aggregation process kinetics and s_0/n_0, estimating aggregates size. Cell factors assays: erythrocyte deformability, by filtration through nucleopore membranes, cell shape, by microscopy, and membrane fluidity by EPR. Plasma: total proteins and CRP, albumin, fibrinogen (Fb), by gravimetry, and IgG and IgM by single radial immuno-diffusion.

Results: AR and C ($x \pm$ SE). $2k_2n_0$: 31.83 $\pm$ 2.84, 23.75 $\pm$ 1.91; s_0/n_0: 0.92 $\pm$ 0.05, 0.87 $\pm$ 0.04. Rigidity index (RI): 14.79 $\pm$ 4.71, 6.92 $\pm$ 1.31. Morphological index: 0.28 $\pm$ 0.03, 0.30 $\pm$ 0.05, n.s. Fb (mg/dl): 382 $\pm$ 80, 299 $\pm$ 70. IgG (mg/dl): 1580 $\pm$ 219, 1296 $\pm$ 158; IgM (mg/dl) 233 $\pm$ 28, 183 $\pm$ 23; albumin (g/dl) 3.84 $\pm$ 0.44, 3.77 $\pm$ 0.51 n.s. $p < 0.05$ accepted. Correlations: $2k_2n_0$ vs. Fb $r = 0.66$; s_0/n_0 vs. Fb $r = 0.51$; $2k_2n_0$ vs. Igs $r = 0.65$; s_0/n_0 vs. Igs $r = 0.56$. $2k_2n_0$ vs. RI $r = -0.59$; s_0/n_0 vs. RI $= -0.52$, $p < 0.05$.

Conclusions: Plasma factors, Igs and Fb increased aggregation, since RI is altered, this reduces the process efficiency regarding aggregation. Patients with active RA present an increased EA, with values modifications associated with the activity index DAS 28-4, thus becoming an RA activity indicator.

Keywords: Rheumatoid arthritis activity, erythrocyte aggregation, DAS 28-4, membrane fluidity, erythrocyte deformability

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1. Introduction

Erythrocytes in static human blood form loose aggregates similar to a stack of coin stacks, such aggregation is referred to as rouleaux formation and is due to their shape, deformability and surface properties.

In saline solutions, erythrocyte stabilizes sterically and do not form rouleaux. For aggregation to occur, requires the presence of macromolecules in the medium, with enough bridging effect to surpass the steric stability of erythrocytes. Within plasma, fibrinogen constitutes the main aggregating protein, assisted by globulins; but albumin, the most abundant plasma protein, is generally regarded as a hindering factor to this phenomenon [1,2].

In physiological conditions, blood flow's shearing effect is enough to produce erythrocyte disaggregation; but in low-flow conditions and in certain pathological situations, increased erythrocyte aggregation can contribute to circulatory disorders and, particularly in the microcirculation, to the occlusions of microvessels.

Erythrocyte aggregation – usually related to blood flow resistance – is increased in different conditions associated to inflammatory processes [3,4] and to rheumatoid arthritis (RA) in particular [5].

Modifications in erythrocyte aggregation in RA patients are regularly studied through the increase in erythrocyte sedimentation rate (ESR) as well as the increase in blood viscosity at low shear rate [6–9].

However, these methods are rough estimations that do no allow assessing whether the cause for such increase lies in cell factors or in the plasma ones [10].

On the contrary, there is no evidence of studies performed assessing cell factors that determine such modification in AR, nor of its tracking in relation to the progress of the disease.

The aim of the present paper is to quantitatively estimate the erythrocyte aggregation kinetic in relation to the activity of the disease; and to analyse the cell factors as well as the plasma ones that might be involved in the erythrocyte aggregation increase, in order to shed light upon how RA affects blood components, and consequently modifies blood fluid behaviour.

2. Materials and methods

2.1. Patients

One hundred female RA patients were included in the present study (mean age 48 ± 17 yrs, range 31–65) attending an outpatient service at the Departamento de Reumatología, Universidad Nacional de Rosario, Argentina.

The patients were part of a follow-up study recruited between 2000 and 2003 [11]. RA diagnosis was established following the American College of Rheumatology criteria (formerly, the American Rheumatism Association) [12,13]. Patients with cardiovascular or liver disease, cancer, chronic infectious diseases, HIV positive serology, diabetes mellitus and heavy smokers ($>$20 cigarettes) and patients who were under medication for RA were excluded.

Disease clinic activity was evaluated by means of the Disease Activity Score (DAS 28-4) [14], through the following equation:

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

where: sqrt(t28) = square root of the number of painful joints from 28 joints; sqrt(sw28) = square root of the number of swollen joints from 28 joints; ln(ESR): natural logarithm of erythrocyte sedimentation

rate in mm/h; GH: general health or patient's global assessment of disease activity on a 0–100 Visual Analogue Scale (VAS).

Cut-off values for DAS 28-4 are as follows: high disease activity > 5.1, remission < 2.6.

At inclusion, all patients received non-steroid anti-inflammatory drugs (NSAIDs).

AR patients were chosen (DAS 28-4 > 5.1), and the modifications in aggregation parameters were studied in relation to the evolution of DAS 28-4 score during 4 years follow-up study.

2.2. Controls

The control group consisted of 40 females non-smoker healthy volunteers, age-matched (mean: 43 ± 12 yrs, range 31–55).

All samples were taken in an ambulatory outpatient setting using the same venepuncture technique from the antecubital fossa with a brachial tourniquet and put into gel activated serum separation tubes: most samples were taken during the morning.

The study protocol was approved by the Ethics Committee of the Facultad de Ciencias Médicas, Universidad Nacional de Rosario, and all participants signed an informed consent according to the recommendations of the Declaration of Helsinki [15].

2.3. Blood sample collection and laboratory assays

Blood samples were obtained by venipuncture from healthy donors and RA patients, and collected in tubes containing EDTA (1.146 mg/ml, Sigma Chemical Co., St. Louis, MO, USA) as anticoagulant.

2.3.1. Determination of erythrocyte aggregation parameters

A specific method is employed, that determines the changes in light transmitted through the blood sample during a stasis term following a disaggregating shakeup. The assessment of such registers allows to obtain two parameters $2k_2n_0$ (characterizing the aggregation process kinetics); and s_0/n_0 (estimates the size of the aggregates [16].

2.4. Cell factors

Erythrocyte filtration was performed in a computerised instrument [17] after Reid et al. technique [18]. 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 cm of H_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):

$$\mathrm{RI} = \frac{T_\mathrm{b} - T_\mathrm{s}}{T_\mathrm{s}} \times \frac{100}{\mathrm{Htc}},$$

where: T_b – time of cell suspension passage through the filter; T_s – time of PBS passage. Htc – haematocrit (10%).

2.4.1. Erythrocyte membrane fluidity

It was determined by electron paramagnetic resonance (EPR) [19], 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 evaluated from the outer hyperfine structures of the spectra, and it was taken as a representative parameter of lipid bilayer rigidity.

2.4.2. Erythrocyte shape

An aliquot of 1% v/v RBC suspension in saline containing 0.25% bovine albumin was placed on a vinyl plastic slide. The cell shape was observed with an inverted microscope, assigning an index according to Bessis Classification [20]. The number of observed cells for each slide is 150, and the informed value is an average of the respective values.

2.4.3. Biochemical assays

Total protein and albumin were measured by colorimetric method, plasma fibrinogen by gravimetry, immunoglobulins G and M by single radial immuno-diffusion technique and rheumatoid factor (RF) by turbidimetry (BioSystems S.A.) and C-reactive protein (CRP) by Singer and Plotz's technique [21].

Eritrosedimentation rate (ESR) was measured according to Westergreen.

2.4.4. 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.

Haematocrit was assessed by microhaematocrit method; haemoglobin concentration by spectrophotometry (cyanmethemoglobin method) and RBC count by manual method (Newbauer camera). From these values, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) was calculated.

2.5. Statistical analysis

Values in RA patients and their controls 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 aggregation parameters ($2k_2n_0$, s_0/n_0) and IgG, IgM, fibrinogen, rigidity index and $T_{//}$.

In the follow-up study, association of erythrocyte aggregation parameters with DAS 28-4 was analysed using rank correlation coefficient (Spearmen's test).

3. Results

Table 1 show the parameters characterizing erythrocyte aggregation. It can be observed that the kinetic rate ($2k_2n_0$) and the aggregate size (s_0/n_0) are increased in erythrocytes from RA patients compared to normal controls ($p < 0.001$). Microscope observation showed that erythrocytes keep their regular shape and volume (see also Table 3).

The concentration level of C-reactive protein, rheumatoid factor, immunoglobulins and fibrinogen (but not albumin) are significantly increased in RA patients in comparison to controls (Table 2).

Table 1

Erythrocyte aggregation parameters in active-RA patients and controls (mean ± standard deviation)

Aggregation parameters*	RA patients ($n = 100$)	Controls ($n = 40$)	p
$2k_2n_0$	31.8 ± 2.8	24.2 ± 1.9	<0.001
s_0/n_0	0.92 ± 0.05	0.85 ± 0.10	<0.001

Comparison performed by Student's t test for unpaired data.

*s_0/n_0 is an indicator of aggregate size, while $2k_2n_0$ estimates aggregation rate.

Table 2

Plasma aggregation promoters factors in RA patients and controls (mean ± standard deviation)

Plasma factors	Patients ($n = 100$)	Controls ($n = 40$)	p
CRP (mg/l)	2.05 ± 1.38	0.50 ± 0.04	<0.01
RF (mUI)	75.51 ± 11.36	14.66 ± 2.06	<0.0000
Total proteins (g/dl)	7.65 ± 0.38	6.89 ± 0.41	<0.01
Albumin (g/dl)	3.84 ± 0.44	3.77 ± 0.51	NS
Igs (g/dl)	2.87 ± 0.25	2.56 ± 0.22	<0.01
Ig G (mg/dl)	1580 ± 219	1296 ± 158	<0.001
Ig M (mg/dl)	233 ± 28	186 ± 23	<0.001
Fibrinogen (mg/dl)	382 ± 80	299 ± 47	<0.01

Comparison performed by Student's t test for unpaired data.

Table 3

Cellular aggregation promoter factors in RA patients and controls (mean ± standard deviation)

Cellular factor	RA patients ($n = 100$)	Controls ($n = 40$)	p
RI	14.79 ± 4.71	6.92 ± 1.31	<0.001
MCV (μm^3)	89.7 ± 1.6	93.15 ± 7.7	NS
Morphological index	0.30 ± 0.05	0.5 ± 0.03	NS
$T_{//}$ (Gauss)	29.13 ± 0.10	29.02 ± 0.20	NS

Comparison performed by Student's t test for unpaired data.

Table 4

Pearson correlation coefficients between aggregation parameters and concentration of fibrinogen, C-reactive protein and immunoglobulins and erythrocyte rigidity index ($n = 100$)

Aggregation parameter	RCP	[Fibrinogen]	[Inmunoglobulins]	RI
$2k_2n_0$	$r = 0.558$	$r = 0.658$	$r = 0.654$	$r = 0.59$
	$p = 0.06$	$p < 0.001$	$p < 0.001$	$p < 0.001$
s_0n_0	$r = 0.45$	$r = 0.51$	$r = 0.56$	$r = 0.52$
	$p = 0.07$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Table 3 shows erythrocyte's mechanical properties related to those appearing in the bibliography as aggregation promoters, such as erythrocyte deformability (estimated through its inverse, the rigidity index RI), membrane fluidity (estimated also by its inverse $T_{//}$), and cell shape. According to the results obtained, erythrocytes from RA patients only differ from normal controls in their lower deformability (higher rigidity index, RI).

Pearson's correlation coefficients between erythrocyte aggregation parameters (s_0/n_0 and $2k_2n_0$) and the concentration of relevant plasma proteins are displayed in Table 4. The correlation between aggregation parameters and the rigidity index is also included. Among the proteins involved in the erythrocyte aggregation process, the correlations are significant only for immunoglobulins and fibrinogen, indicating that the increase in erythrocyte aggregation noticed on active-RA patients depends mainly on them and not on CRP.

Note that a negative significant correlation is found between the aggregation parameters and RI, indicating that *increased* rigidity leads to *decreased* aggregation rate and aggregate size. No significant correlations were found between the erythrocyte aggregation parameters and membrane fluidity ($p > 0.05$), cell volume ($p > 0.05$) nor internal viscosity (estimated by MCHC) ($p > 0.05$) (data not shown). Taken

Table 5

Association between the erythrocyte aggregation parameters with DAS 28-4 in the follow-up study ($n = 16$)

	Inclusion	1 yr	2 yr	3 yr	4 yr
$2k_2n_0$	0.84*	0.96*	0.90*	0.91*	0.94*
s_0n_0	0.81*	0.93**	0.89**	0.87**	0.92**

Rank correlation coefficient (Spearmen's test) r_s statistical significance $^*p < 0.05$; $^{**}p < 0.01$.

together, these data indicate that cell properties are not related to the increase in aggregation tendency in RA.

Table 5 shows the association between the erythrocyte aggregation parameters with DAS 28-4 on 16 patients during the 4-yrs follow-up. DAS 28-4 decreased steadily along this time, and it can be seen that the aggregation parameters stayed highly correlated with the disease score.

4. Discussion

Erythrocyte aggregation depends on both its mechanical properties and on its surface characteristics. It takes place in the presence of macromolecules able to trigger such phenomenon in the medium [4,22,23].

The erythrocyte disaggregation is considered vital for the normal perfusion of the tissues [24]. In physiological conditions, the blood flow shear keeps the cells disaggregated; however, in low-flow conditions and in certain pathological situations, increased erythrocyte aggregation can lead to circulatory disorders and – in microcirculation – to the occlusion of the microvessels as well as to tissue hypoxemia [25–28].

Along with the known ESR increase [3,7,9,11], our results show that RA-active patients present a significant increase in erythrocyte aggregation. Moreover, a follow-up study of the treated patients showed that, in remission, aggregation monotonically decreases with the activity index (DAS 28-4) (Table 5).

Several factors could have a relevant role in increased erythrocyte aggregation. Specifically, active RA appears accompanied by raised CRP, immunoglobulins and fibrinogen concentrations [3,8,11].

Many reports deal with the role played by fibrinogen and CRP, as well as several inflammation-marker proteins such as IgG, IgM, IgA and ceruplasmine in the induction and maintenance of increased erythrocyte aggregation in the blood of RA patients [29].

In a former paper [11] we proposed that in RA, a widespread cell membrane damage is expressed in impaired erythrocyte deformability, turning haemorheological parameters into reliable tools to study disease evolution.

In the present paper we corroborate our assumption that only the rigidity index showed modifications, given that cell volume, mean corpuscular haemoglobin concentration and membrane fluidity did not reveal significant differences from controls.

The significant negative correlation between RI and aggregation parameters indicates that the diminution in erythrocyte deformability do not favour aggregation. This unexpected result leads us to conclude that plasmatic factors play a predominant role in the increase of erythrocyte aggregation in RA patients. Besides, the assessment of plasma proteins and their correlation with the s_0n_0 and $2k_2n_0$ parameters showed that the increase in erythrocyte aggregation in these patients is more closely related to the increased levels of immunoglobulins ($p < 0.001$) and fibrinogen ($p < 0.001$), than to CRP.

In conclusion, the present work shows that patients with active RA present an increased erythrocyte aggregation, whose value modifies along with the activity index DAS 28-4. This fact makes aggregation parameters reliable rheumatoid arthritis activity indicators.

From the assessment of the intervening factors, the results obtained demonstrate that plasma factors – immunoglobulins and fibrinogen in particular – are the determiners of the increased aggregation, given that the cell factor altered, RI, reduces the process' efficacy in rate and size of the aggregates.

References

[1] S. Chien, Aggregation of red blood cells. Energy balance at the interface, *Ann. N.Y. Acad. Sci.* **27** (1983), 103–104.

[2] T.V. Korotaeva, N.N. Firsov, A. Bjelle and M.A. Vishlova, Erythrocytes aggregation in healthy donors at native and standard hematocrit: The influence of sex, age, immunoglobulins and fibrinogen concentrations. Standardization of parameters, *Clin. Hemorheol. Microcirc.* **36**(4) (2007), 335–343.

[3] X. Weng, G. Cloutier, R. Beaulieu and G.O. Roederer, Influence of acute-phase proteins on erythrocyte aggregation, *Am. J. Physiol. Heart Circ. Physiol.* **271** (1996), H2346–H2352.

[4] R. Ben Ami, G. Barstein, D. Zeltser et al., Parameters of red blood cell aggregation as correlates of the inflammatory state, *Am. J. Physiol. Heart Circ. Physiol.* **280** (2001), H1982–H1988.

[5] P. Halberg, *Rheumatoid Arthritis. History in Rheumatology*, J.H. Klipped and P.A. Dieppe, eds, Mosby, London, 1997, pp. 1–13.

[6] E. Ernst, W. Thies, A. Matrai, N. Seichert, P. Schöps, I. Magyarosy, A. Kraft-Kupfer and M. Schattenkirchner, Hemorheologic abnormalities in chronic arthritis, *Clin. Hemorheol.* **7** (1987), 591–598.

[7] M. Gudmundsson and A. Bjelle, Plasma viscosity in the monitoring of therapy in rheumatoid arthritis patients, *Scand. J. Rheumatol.* **24**(4) (1995), 219–224.

[8] M. Gudmundsson, A. Oden and A. Bjelle, On whole blood viscosity measurements in healthy individuals and in rheumatoid arthritis patients, *Biorheology* **31**(4) (1994), 407–416.

[9] C.A. Kelly, J. McClelland, B. Fail and D. Walker, Erythrocyte sedimentation rate, plasma and serum viscosity as measures of disease activity in rheumatoid arthritis, *Br. J. Rheumatol.* **26**(2) (1987), 136–138.

[10] A. Pribush, L. Hatzkelson, D. Meyerstein and N. Meyerstein, A novel technique for quantification of erythrocyte aggregation abnormalities in pathophysiological situations, *Clin. Hemorheol. Microcirc.* **36**(2) (2007), 121–132.

[11] A. Luquita, L. Urli, A. Dominighini, M.J. Svetaz, A.M. Gennaro, R. Volpintesta, S. Palatnik and M. Rasia, Haemorheological variables as a rheumatoid arthritis activity indicator, *Clin. Hemorheol. Microcirc.* **30** (2004), 9–17.

[12] F.C. Arnett, S.M. Edworthy, D.A. Bloch et al., The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis, *Arthrit. Rheum.* **31** (1988), 315–324.

[13] B.J. Harrison, D.P.M. Symmons, E.M. Barrett and A.J. Silman, The performance of the 1987 ARA classification criteria for rheumatoid arthritis in a population based cohort of patients with early inflammatory arthritis, *J. Rheumatol.* **25** (1998), 2324–2330.

[14] D.M. Van der Heijde and J.W. Jacobs, The original "DAS" and the "DAS28" are not interchangeable. Comment on the articles by Prevoo et al., *Arthrit. Rheum.* **41**(5) (1998), 942–945.

[15] The International Response to Helsinki VI, The WMA's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, as adopted by the 52nd WMA General Assembly, Edinburgh, October 2000.

[16] S.M. Bertoluzzo, A. Bollini, M. Rasia and A. Raynal, Kinetic model for erythrocyte aggregation, *Blood Cells Mol. Dis.* **25**(22) (1999), 338–348.

[17] Y. Kikuchi, T. Arat and T. Koyarna, Improved filtration method for red cell deformability measurement, *Med. Biol. Eng. Comput.* **42** (1983), 127–135.

[18] H.L. Reid, A.J. Barnes, P.J. Lock, J.A. Dormandy and T.L. Dormandy, A sample method for measuring erythrocyte deformability, *J. Clin. Pathol.* **29** (1976), 855–858.

[19] I.D. Campbell and D.A. Dwek, *Biological Spectroscopy*, Benjamin/Cummings, Menlo Park, CA, 1984, pp. 196–197.

[20] M. Bessis, in: *Red Cell Shape. Physiology, Pathology, Ultrastructure*, Springer-Verlag, Heidelberg, 1973, pp. 1–23.

[21] J.M. Singer, C.M. Plotz, E. Parker and S.K. Elster, The latex fixation test, *Am. J. Clin. Path.* **28** (1957), 611.

[22] M.L. Melamies, H.M. Ruutsalo and H. Nissila, Evaluation of a quantitative immunoturbidimetric assay for rheumatoid factors, *Clin. Chem.* **32** (1986), 1890–1894.

[23] G.B. Nash, S. Wenby, O. Sowememo-Coker and H.J. Meiselman, Influence of cellular properties on red cell aggregation, *Clin. Hemorheol.* **7** (1987), 93–108.

[24] M.W. Rampling, The great divide: From viscometer to vasculature, *Clin. Hemorheol. Microcirc.* **39** (2008), 9–20.

[25] T.L. Fabry, Mechanism of erythrocyte aggregation and sedimentation, *Blood* **70** (1987), 1572–1576.

[26] M. Soutani, Y. Suzuki, N. Tateishi and N. Maeda, Quantitative evaluation of flow dynamics of erythrocytes in microvessels: influence of erythrocyte aggregation, *Am. J. Physiol. Heart Circ. Physiol.* **268** (1995), H1959–H1965.

[27] G. Mchedlishvili, M. Varazashvili and L. Gobejishvili, Local RBC aggregation disturbing blood fluidity and causing stasis in microvessels, *Clin. Hemorheol. Microcirc.* **26** (2002), 99–107.

[28] M. Cabel, H.J. Meiselman, A.S. Popel and P.C. Johnson, Contribution of red blood cell aggregation to venous vascular resistance in skeletal muscle, *Am. J. Physiol. Heart Circ. Physiol.* **272** (1997), H1020–H1032.

[29] T.G. Varazashvili and M.G. Mc Hedlishvili, Local hemorheological disorders during chronic inflamation, *Clin. Hemorheol. Microcirc.* **30**(3/4) (2004), 427–429.