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Erythrocyte damage in mild and severe psoriasis

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Summary

Background Psoriasis is a common chronic and recurrent inflammatory skin disorder. Oxygen metabolites and proteases released by activated inflammatory cells may induce oxidative and proteolytic damage to plasma constituents and red blood cells (RBCs). RBCs have a limited biosynthesis capacity and poor repair mechanisms.

Objectives To study RBCs as a potential cumulative marker of oxidative and proteolytic stress in psoriasis, and as a marker of worsening of the disease.

Methods The study was performed in 70 patients with mild or severe psoriasis and in 40 control individuals. We evaluated total and differential leucocyte count and, as markers of leucocyte activation, plasma elastase and lactoferrin. Besides the basic RBC study (RBC count, haematocrit, haemoglobin concentration and haematimetric indices) we evaluated antioxidant defences (catalase, superoxide dismutase, glutathione peroxidase and selenium), osmotic fragility and reticulocyte count; in the RBC membrane we evaluated lipid peroxidation and susceptibility to lipid peroxidation, membrane fluidity, levels of cholesterol and phospholipids, membrane-bound haemoglobin, band 3 profile and levels of vitamin E; serum levels of bilirubin, total plasma antioxidant capacity, lipid profile and lipid peroxidation were also evaluated.

Results Psoriasis patients showed a rise in leucocytes, mainly neutrophils, which was associated with a rise in elastase and lactoferrin. Patients had a reduced RBC count, antioxidant defences and membrane fluidity, elevated membrane lipid peroxidation, membrane-bound haemoglobin, osmotic fragility and reticulocyte count, and a different band 3 profile. Most of these modifications were enhanced in severe psoriasis.

Conclusions In summary, our data show that the RBCs are at a lower number in psoriasis patients, and present several changes denoting an enhanced damage and/or ageing process, which seem to be strongly connected with neutrophil activation, oxidative stress and worsening of psoriasis.

Key words: antioxidants, erythrocyte, neutrophil, oxidative stress, psoriasis

Psoriasis is a chronic and recurrent inflammatory skin disease, known as an oxidative stress condition.[1,2] As with any inflammatory disease, psoriasis often presents a rise in leucocytes, namely in neutrophils, and a reduction in total red blood cells (RBCs). Psoriasis lesions that are clinically active show infiltration of leucocytes, and several studies report high levels of leucocyte activation products in the peripheral blood of these patients.[3,4] Activated white blood cells (WBCs) are important sources of reactive oxygen species (ROS)

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and proteases, either of which may impose oxidative and proteolytic changes to plasma constituents and to circulating RBCs.[5–12] Several enzymes are involved in the RBC antioxidant defence, such as catalase (Cat), superoxide dismutase (SOD) and glutathione peroxidase (GPx), which is a selenium (Se)-dependent enzyme. The levels of reduced glutathione (GSH) in the cell are also important in protecting the cell from the deleterious action of ROS. The levels of lipid-soluble membrane vitamin E are important in the defence against membrane lipid peroxidation.[13]

By diffusing across the cell membrane, oxygen metabolites may trigger an oxidative stress within the cells. ROS usually trigger an upregulation of the antioxidant defences; however, an oxidative stress will develop when the antioxidant capacity of the cell is overwhelmed.

Presenting a limited biosynthesis capacity, the circulating erythrocyte suffers and accumulates physical and chemical changes, which become more pronounced with cell age, and whenever an unusual physical or chemical stress develops.[14,15] RBCs that develop intracellular defects earlier during their life span are removed from circulation at an earlier stage. Several RBC studies have shown that oxidation and/or proteolysis plays a relevant role in the erythrocyte ageing process. Several modifications are known to occur throughout the life span of the circulating RBCs, namely a reduction in cell volume and in enzymatic activity, leading to a reduction in the metabolic activity and in the antioxidant defences of the cell.

The removal of senescent or damaged RBCs seems to involve the development of a neoantigen on the plasma membrane surface, marking the cell for death.[16] This neoantigen is immunologically related to band 3, a RBC transmembrane protein.[17] The degradation of the metabolism and of the antioxidant defences, by favouring the development of oxidative stress, allows the oxidation and linkage of haemoglobin (Hb) to band 3, promoting its aggregation and the binding of natural antiband 3 autoantibodies[18] which, together with complement, mediate the removal of RBCs.[18,19]

Several markers of oxidative stress have been studied in psoriatic RBCs, namely the activity of antioxidant enzymes, membrane lipid peroxidation and membrane fluidity. Recently, changes in the domain structure of those RBCs, namely in the portion of the most ordered domain, were also reported as potential markers of oxidative RBC damage, and additionally as markers for the activity of psoriasis.[20,21]

Because leucocyte activation seems to be associated with psoriasis and with worsening of the disease,[4] we wondered if the release of ROS and proteases, by imposing oxidative and proteolytic changes to RBCs, may lead to an accelerated RBC ageing and premature removal. The aim of our work was to clarify the frequent association of psoriasis with a reduction in total RBCs, and to look for a correlation between this reduction and worsening of the disease. For this purpose, we studied a group of psoriasis patients with mild or severe psoriasis and a group of controls.

We evaluated total and differential leucocyte count and, as markers of leucocyte activation, plasma elastase and lactoferrin. Besides the basic RBC study [RBC count, haematocrit (Ht), Hb concentration and haematimetric indices] we evaluated the antioxidant defences (Cat, SOD, GPx and Se), osmotic fragility and reticulocyte count; in the RBC membrane we evaluated lipid peroxidation and susceptibility to lipid peroxidation, membrane fluidity, levels of cholesterol (Chol) and phospholipids (Phlip), membrane-bound Hb (MBH), the band 3 profile and levels of vitamin E; serum levels of bilirubin (as an index of RBC removal), total plasma antioxidant capacity, plasma lipid profile and lipid peroxidation were also evaluated.

Materials and methods

Subjects

The protocol used was approved by the Committee on Ethics of the University Hospital of Coimbra (Portugal), and all the patients and controls gave their consent. The control group included 40 apparently healthy adults [55% men and 45% women, mean ± SD age 47·4 ± 13·3 years, with a mean ± SD body mass index (BMI) of 24·4 ± 1·8 kg m^{-2}], with no history of any skin disease, and presenting with normal haematological and biochemical values. Alcoholics were excluded from the study, as well as individuals presenting with other associated diseases, namely anaemia, diabetes mellitus, cardiovascular, liver or kidney diseases.

The patient group comprised 70 adults (57% men and 43% women, mean ± SD age 45·9 ± 12·2 years and BMI 24·6 ± 2·9 kg m^{-2}) with mild or severe psoriasis vulgaris. The disease was diagnosed from 0·5 to 50 years before this study. Disease severity was graded according to the Psoriasis Area and Severity Index (PASI) at the time of blood collection.[22] About half of the

patients had severe psoriasis or active psoriasis (AP) (PASI > 3), and the other half of the patients had mild psoriasis or inactive psoriasis (IP) (PASI < 3).

None of the patients had received any systemic or local steroid medication or any phototherapy treatment for at least 1 month prior to blood collection. In addition, the controls, as well as the patients, were not receiving any kind of medication, namely antioxidants, vitamins or methotrexate. Moreover, we evaluated in controls and patients the serum levels of vitamin B_{12}, folic acid and iron, and excluded all individuals showing reduced values.

Collection and preparation of blood samples

Following fasting of the subjects for 12 h, blood was collected with and without anticoagulant [ethylenediamine tetraacetic acid (EDTA) and heparin as anticoagulants], in order to obtain whole blood, plasma and serum. None of the collected samples was icteric or haemolysed.

To isolate RBCs and prepare their membranes,[23–25] whole blood (EDTA as anticoagulant) was centrifuged at 2300 *g* for 10 min at 4 °C. The plasma was processed for biochemical studies, the buffy coat was discarded and the isolated RBCs were washed three times in saline; afterwards, they were lysed by using a hypotonic solution (Tris–HCl 1 mmol L^{-1}, K_2 EDTA 1·44 mmol L^{-1}, pH 7·4). After 15 min under moderate agitation, the haemolysates were centrifuged at 20 000 *g* for 10 min at 4 °C. The RBC membrane pellet obtained was carefully washed with aisotonic buffer (Tris–HCl 1 mmol L^{-1}, K_2 EDTA 1·44 mmol L^{-1}, NaCl 17 mmol L^{-1}, pH 7·4) until almost complete removal of Hb. The last wash was performed by using a buffer solution of Tris–HCl 10 mmol L^{-1}, pH 7·4. The RBC membranes were resuspended in this buffer solution, immediately and rapidly frozen by submerging the tubes with the membrane suspensions in liquid nitrogen, and stored at − 80 °C until assayed. These RBC membrane suspensions were used for all membrane study estimations except for the band 3 profile and the MBH. For these, plasma and leucocytes were separated from RBCs after centrifugation on a double density gradient (Histopaque 1·077 and 1·119; Sigma, St Louis, MO, U.S.A.). The RBCs were washed with saline and immediately lysed, according to the method of Dodge *et al.*[26] The membranes were washed in Dodge buffer (the first two washes used a protease inhibitor, phenylmethylsulphonyl fluoride, at a final concentration of 0·1 mmol L^{-1} in Dodge buffer) and frozen at − 80 °C until assayed. The protein concentration of the membrane suspensions was determined.[27]

Qualitative and quantitative red blood cell studies

Whole blood (EDTA as anticoagulant) was used for haematological procedures. RBC and WBC count, Ht, Hb concentration and the haematimetric indices [mean cell volume (MCV), mean cell Hb (MCH) and mean cell Hb concentration (MCHC)] were measured using an automatic blood cell counter (Autocounter AC 970).

To evaluate the concentration of reticulocytes, a few drops of the blood sample were taken and added to an equal volume of brilliant cresyl blue solution (1% in saline). After incubation at room temperature (10 min), the percentage of reticulocytes was evaluated in a blood smear and the respective concentration calculated.

Erythrocyte osmotic fragility

The osmotic fragility test was performed according to a previously described method.[28] Briefly, saline solutions buffered to pH 7·4 were used at different concentrations (1·0–9·0 g L^{-1}); heparinized blood was added to these solutions in the proportion 1 : 100 (50 μL blood in 5 mL of buffered solution); haemolysis was evaluated after 30 min of incubation at room temperature by measuring the absorbance at 540 nm of the supernatant obtained after centrifugation (1500 *g* for 5 min). The absorbance of the isotonic RBC suspension was used as the zero haemolysis, and the absorbance of the most hypotonic solution as the total haemolysis. The results were expressed as the concentration of the hypotonic buffered saline in which we observed 50% haemolysis.

Erythrocyte antioxidant defences

Heparinized blood was used to obtain the RBCs for the evaluation of the enzymatic activities of Cat, SOD and GPx, and the levels of GSH and Se. In the case of GPx, the study was performed in whole blood, as its plasmatic activity is extremely low when compared with the RBC activity.[29]

Whole blood was centrifuged, the buffy coat was discarded, and the isolated RBCs were washed in saline. The RBC pellet was then used to evaluate Cat and SOD activity. The activities of SOD and GPx were evaluated by using commercially available kits (RANSOD and RANSEL, respectively, from Randox, Crumlin, U.K.).

The enzymatic activity of Cat was evaluated by using a spectrophotometric method.[30] All the spectrophotometric analyses used an automatic analyser Hitachi 704 (Boehringer Mannheim, Mannheim, Germany). The enzymatic activities were expressed as IU g^{-1} Hb. The erythrocyte GSH levels (μmol g^{-1} Hb) were evaluated according to the method of Beutler *et al.*[31]

Hydride generation followed by atomic absorption spectroscopy was used to determine the concentrations of Se in whole blood and plasma, following digestion of the samples with nitric and sulphuric acids.[32] We then estimated the RBC Se concentration by calculating the difference between the whole blood and plasma values obtained (data not shown).

Erythrocyte membrane studies

Erythrocyte membrane lipid peroxidation and membrane susceptibility to lipid peroxidation Membrane lipid peroxidation was estimated by thiobarbituric acid (TBA) reactivity (TBA assay).[33] To evaluate the RBC membrane susceptibility to lipid peroxidation, we evaluated the TBA reactive species of the membranes, after an *in vitro* stress imposed on RBCs by hydrogen peroxide (2 h at 37 °C).[34] This RBC membrane susceptibility to lipid peroxidation was also evaluated by the parinaric acid (PnA) assay.[35] Briefly, the oxidation of 1·5 μmol L^{-1} PnA incorporated in RBC ghosts (200 μmol L^{-1} in Phlip) suspended in 10 mmol L^{-1} Tris-maleate, 150 mmol L^{-1} NaCl, pH 7·4, was induced by free radicals generated with the Fe^{2+}/ascorbate system (1 μmol L^{-1}/2 μmol L^{-1}), at 37 °C. The incorporation of the fluorescent probe PnA was achieved by incubating an aliquot of an ethanolic solution (2 mmol L^{-1} PnA) with RBC ghosts for 2 min before starting the oxidation reactions. The degradation was monitored by detecting the decay of fluorescence in a Perkin-Elmer LS-50 spectrofluorometer (Norwalk, U.S.A.) with the excitation wavelength at 342 nm and the emission at 413 nm. The excitation and emission slit widths were 5 and 5·5 nm, respectively.[36] Control experiments were performed to monitor the spontaneous peroxidation of PnA in the absence of the inducer system. The results are expressed as the slope of fluorescence.

Erythrocyte membrane fluidity Erythrocyte membrane fluidity was determined by using the hydrophobic fluorescent probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and its cationic derivative 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) (Molecular Probes, Junction City, OR, U.S.A.).

To perform the fluorescence polarization measurements,[37,38] the RBC membranes were dispersed in 50 mmol L^{-1} KCl, 10 mmol L^{-1} Tris-maleate, pH 7·0. TMA-DPH and DPH, from a 2-mmol L^{-1} stock solution in tetrafuran, were incorporated into membrane suspensions (345 μmol L^{-1} in Phlip), in order to obtain a lipid/probe molar ratio of about 400. The prepared suspensions were incubated at 37 °C for 2 h. The fluorometric measurements were carried out with a Perkin-Elmer LS-50 computer-controlled spectrofluorometer. In the studies with both probes, the degree of fluorescence polarization was determined as reported elsewhere,[39] excepting that the excitation and emission slits were 3 and 4 nm, respectively. Appropriate control experiments were carried out without added probes to correct for the contribution of light scattering.

Erythrocyte membrane phospholipids and cholesterol As an indirect marker of RBC membrane fluidity we evaluated the levels of membrane Chol and Phlip, as well as their ratio. Lipids were extracted from RBC membranes by chloroform/methanol (2 : 1 v/v), followed by chloroform/methanol/H_2O (30 : 60 : 20 v/v/v), according to Alling *et al.*[40] Chol content was assayed enzymatically using Chol oxidase.[41] Phlip content was determined as inorganic phosphate by the method of Bartlett,[42] after hydrolysis at 180 °C in perchloric acid 70% (v/v).[43]

Erythrocyte membrane antioxidant defences Erythrocyte membrane vitamin E was evaluated by high-performance liquid chromatography. Washed RBCs (500 μL) were mixed with sodium dodecyl sulphate (SDS; 1·5 mL, 10 mmol L^{-1}), 2 mL of ethanol and 2 mL of *n*-hexane. We then used a vortex-type mixer to extract vitamin E, by mixing for about 5 min. After centrifugation at 3200 ***g*** for 5 min, we used 1 mL of the organic phase, which was evaporated to dryness under a gentle stream of nitrogen. These dry extracts, frozen at − 80 °C until assayed, were dissolved in 250 μL of an α-tocopherol solution (100 μg dL^{-1} in *n*-hexane).

The extracts were chromatographed in *n*-hexane/methanol (99·1 : 0·9 v/v) at a flow rate of 1·5 mL min^{-1}, by using a reversed-phase column (Spherisorb S10W 250 × 4·6 mm) with ultraviolet detection at 287 nm (HP 1100; Bio-Rad, Hercules, CA, U.S.A.). The subsequent quantitative determination was then performed with the help of an internal standard and the results expressed as nmol g^{-1} Hb.

Membrane-bound haemoglobin MBH was measured spectrophotometrically, after membrane protein dissociation with Triton X-100 (5% in Dodge buffer); the

absorbance was measured at 415 nm, and this value was corrected by the background absorbance at 700 nm; the value obtained and the protein concentration were used to estimate percentage MBH.

Erythrocyte membrane band 3 profile RBC membrane suspensions were treated with an equal volume of a solubilization buffer containing 0·125 mol L^{-1} Tris–HCl pH 6·8, 4% SDS, 20% glycerol and 10% 2-mercaptoethanol, heat denatured and submitted to SDS–polyacrylamide gel electrophoresis (20 μg protein per lane), using the discontinuous Laemmli system (a 9% separating gel and a 4·5% stacking gel).[44] Membrane proteins were electrophoretically transferred from SDS gels to a nitrocellulose sheet with a porosity of 0·2 μm (Sigma).[45] Additional reactive sites on the nitrocellulose were blocked by incubation in 3% gelatine and 0·1% Triton-X 100 in phosphate-buffered saline (PBS) pH 7·0 for 1 h at room temperature and under gentle rotation. Band 3 immunoblot was performed:[46] antihuman band 3 monoclonal antibodies, produced in mouse, recognizing an epitope located in the cytoplasmic pole of the band 3 molecule[47] (Sigma) were added (dilution 1 : 3000) and incubated for 4 h; washing of the nitrocellulose was followed by addition and incubation with peroxidase-linked antimouse IgG (Vector Laboratories, Burlingame, CA, U.S.A.) for 1 h (dilution 1 : 4000). The incubations were carried out at room temperature; the antibody dilutions were prepared with PBS pH 7·0 containing 0·1% Triton-X 100 and 0·5% gelatine. In the washes, the same buffer without gelatine was used. Hydrogen peroxide and horseradish peroxidase colour developer reagent were used to develop the immunoblot. The band 3 profile was quantified by densitometry (Cybertech CS1); we measured the percentage of each band, i.e. the percentages of high molecular weight aggregates (HMWAg), band 3 monomer and proteolytic fragments (Pfrag).

Further assays

Leucocyte activation products Plasma concentration of polymorphonuclear elastase and lactoferrin were evaluated by enzyme immunoassays (PMN Elastase immunoassay, Merck, Darmstadt, Germany and Bioxytech Lactof enzyme immunoassay, Oxis International, Portland, OR, U.S.A., respectively).

Oxidative stress Total plasma antioxidant capacity was evaluated by a colorimetric assay (TAS, Randox). Plasma lipid peroxidation was estimated by TBA assay.[33]

Serum bilirubin Total serum bilirubin concentration was evaluated by using a commercially available kit (BIL-T; Roche, Basel, Switzerland).

Lipid profile Serum was used to study the lipid profile, which included the evaluation of total Chol, triglycerides (TG), high-density lipoprotein Chol (HDLc), low-density lipoprotein Chol (LDLc), very low-density lipoprotein Chol (VLDLc) and Phlip. Total Chol, TG and Phlip concentrations were measured enzymatically by using commercially available kits (Roche) on an autoanalyser (Hitachi 704). HDLc was evaluated by using the method described for total Chol, after precipitation of the lipoproteins containing apolipoprotein B [LDL, VLDL and lipoprotein (a)] by a phosphotungstic acid/magnesium chloride mixture. LDLc was computed by Friedwald's equation.[48]

Statistical analysis

The statistical analysis was performed by using the SPSS package. To evaluate differences between groups, we used Student's *t*-test for the determinations presenting a Gaussian distribution, and the Mann–Whitney test for those presenting a non-Gaussian distribution. $P < 0{\cdot}05$ was considered statistically significant. The measurements are expressed as mean ± SD. The strength of the association between the parameters was estimated by the Pearson correlation coefficient.

Results

All the results were analysed in two ways, on the one hand to study the differences between healthy controls and psoriasis patients, and on the other to evaluate the changes according to the activity of the disease (mild vs. severe psoriasis).

Qualitative and quantitative RBC studies (controls and psoriasis patients) are presented in Table 1, as are percentage and total reticulocytes, as markers of bone marrow production, and the plasma levels of total bilirubin, as a marker of RBC removal and destruction. When compared with the control we found that total psoriasis patients (IP + AP) had significantly lower values for RBCs ($P < 0{\cdot}001$), Ht ($P < 0{\cdot}001$) and Hb ($P < 0{\cdot}001$). The haematimetric indices showed that the RBCs from psoriasis patients were smaller ($P < 0{\cdot}05$) and with a reduced MCH ($P < 0{\cdot}01$), although the MCHC did not change. The percentage and total reticulocytes were significantly higher in patients ($P < 0{\cdot}001$ and $P < 0{\cdot}05$, respectively). The

Table 1. Qualitative and quantitative red blood cell (RBC) study (mean ± SD) for controls and psoriasis patients

	Controls ($n = 40$)	IP + AP ($n = 70$)	*P*-value (IP + AP)/ controls	IP ($n = 40$)	*P*-value IP/controls	AP ($n = 30$)	*P*-value AP/controls	*P*-value IP/AP
Age (years)	47·4 ± 13·3	45·9 ± 12·2	NS	46·2 ± 13·0	NS	45·6 ± 11·4	NS	NS
BMI (kg m^{-2})	24·4 ± 1·8	24·6 ± 2·9	NS	24·1 ± 3·2	NS	25·2 ± 2·5	NS	NS
Smokers	17 (42·5%)	28 (40%)		15 (37·5%)		13 (43%)		
RBCs ($\times 10^{12}$ L^{-1})	4·91 ± 0·21	4·65 ± 0·19	< 0·001	4·70 ± 0·18	< 0·001	4·59 ± 0·19	< 0·001	< 0·05
Haematocrit (L L^{-1})	0·46 ± 0·03	0·43 ± 0·02	< 0·001	0·44 ± 0·02	< 0·001	0·42 ± 0·02	< 0·001	< 0·01
Haemoglobin (g dL^{-1})	14·6 ± 0·9	13·6 ± 0·7	< 0·001	13·8 ± 0·6	< 0·001	13·4 ± 0·7	< 0·001	< 0·01
MCV (fL)	94·4 ± 4·1	92·6 ± 2·2	< 0·05	92·8 ± 2·5	< 0·05	92·3 ± 1·9	< 0·01	NS
MCH (pg)	29·8 ± 0·7	29·3 ± 0·9	< 0·01	29·4 ± 0·9	NS	29·2 ± 0·9	< 0·01	NS
MCHC (g dL^{-1})	31·6 ± 1·4	31·7 ± 1·1	NS	31·7 ± 1·1	NS	31·6 ± 1·2	NS	NS
OF (NaCl solution: g L^{-1})	4·44 ± 0·04	4·48 ± 0·04	< 0·001	4·46 ± 0·03	< 0·01	4·50 ± 0·05	< 0·001	< 0·01
% Reticulocytes	1·19 ± 0·24	1·36 ± 0·16	< 0·001	1·30 ± 0·13	< 0·01	1·43 ± 0·16	< 0·001	< 0·001
Reticulocytes ($\times 10^{9}$ L^{-1})	58·0 ± 11·2	62·9 ± 7·5	< 0·05	61·2 ± 6·6	NS	65·2 ± 8·0	< 0·01	< 0·05
Bilirubin (mg dL^{-1})	0·69 ± 0·12	0·76 ± 0·11	< 0·01	0·75 ± 0·10	< 0·05	0·76 ± 0·12	< 0·05	NS

IP, inactive psoriasis; AP, active psoriasis; BMI, body mass index; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; OF, osmotic fragility; NS, nonsignificant.

total bilirubin level was also significantly increased in psoriasis patients ($P < 0·01$). The RBCs from patients presented a significantly increased osmotic fragility ($P < 0·001$).

When comparing the values for IP patients with those for controls we found the same changes as for total patients, with the exception of MCH and total reticulocytes. In AP patients, we found the same changes as for total patients. Most of the parameters studied showed more pronounced changes in AP patients than in IP patients, when compared with controls. In severe psoriasis we found more reduced values for RBCs, Ht, Hb, MCV and MCH, and more increased values for reticulocytes (percentage and total), bilirubin and osmotic fragility (Table 1).

When studying the values according to the severity of psoriasis, i.e. comparing the values for IP patients with those for AP patients (Table 1), we found that most changes were enhanced significantly with worsening of the disease.

The evaluation of total and differential WBC count (Table 2) showed a significant rise in total WBC in patients ($P < 0·01$), resulting from a significant increase in neutrophils ($P < 0·001$) and lymphocytes ($P < 0·05$); for monocytes we found a significant reduction ($P < 0·001$). A significant reduction in the RBC/neutrophil ratio was also observed ($P < 0·001$). The rise in neutrophils seems to be associated with their activation, as shown by the significant rise in elastase ($P < 0·001$) and lactoferrin ($P < 0·001$).

When comparing patients with mild psoriasis with controls (Table 2), we found that, despite a similar value for total WBCs, there was a significant rise in neutrophils ($P < 0·001$) and a significant reduction in monocytes ($P < 0·001$), associated with significantly higher values for elastase ($P < 0·001$) and lactoferrin ($P < 0·001$).

In severe psoriasis, the rise in total WBCs ($P < 0·001$) was significant when compared with controls (Table 2), and this rise was linked to significant rises in neutrophils ($P < 0·001$) and lymphocytes ($P < 0·001$), as well as to significant rises in elastase ($P < 0·001$) and lactoferrin ($P < 0·001$).

Concerning the severity of disease (IP vs. AP), we found in severe psoriasis significantly higher values for total and differential WBC count, associated with significantly higher levels of leucocyte activation products (Table 2).

The evaluation of erythrocyte antioxidant defences (Table 3) showed that psoriasis patients had a reduced RBC antioxidant capacity, as shown by a significant reduction in membrane vitamin E ($P < 0·05$), GPx activity ($P < 0·01$), Se ($P < 0·05$) and GSH ($P < 0·001$). No significant differences were found for Cat and SOD. In mild psoriasis the same tendency was observed, although significant differences were only

Table 2. Total and differential white blood cell (WBC) count and plasma neutrophil activation products, elastase and lactoferrin (mean ± SD), for controls and psoriasis patients

	Controls ($n = 40$)	IP + AP ($n = 70$)	*P*-value (IP + AP)/controls	IP ($n = 40$)	*P*-value IP/controls	AP ($n = 30$)	*P*-value AP/controls	*P*-value IP/AP
WBCs ($\times 10^9$ L^{-1})	6·69 ± 1·30	7·59 ± 1·51	< 0·01	6·93 ± 1·28	NS	8·46 ± 1·37	< 0·001	< 0·001
Neutrophils ($\times 10^9$ L^{-1})	3·93 ± 0·77	5·15 ± 1·02	< 0·001	4·65 ± 0·81	< 0·001	5·81 ± 0·89	< 0·001	< 0·001
Lymphocytes ($\times 10^9$ L^{-1})	2·32 ± 0·45	2·53 ± 0·49	< 0·05	2·35 ± 0·43	NS	2·78 ± 0·47	< 0·001	< 0·001
Monocytes ($\times 10^9$ L^{-1})	0·24 ± 0·05	0·21 ± 0·05	< 0·001	0·19 ± 0·03	< 0·001	0·23 ± 0·05	NS	< 0·001
RBC/neutrophils	1290 ± 238 ($n = 40$)	933 ± 204 ($n = 60$)	< 0·001	1033 ± 199 ($n = 30$)	< 0·001	801 ± 118 ($n = 30$)	< 0·001	< 0·001
Age (years)	47·4 ± 13·3	46·1 ± 12·2	NS	46·6 ± 13·4	NS	45·6 ± 11·4	NS	NS
BMI (kg m^{-2})	24·4 ± 1·8	24·7 ± 3·1	NS	24·2 ± 3·6	NS	25·2 ± 2·5	NS	NS
Smokers	17 (42·5%)	25 (42%)		12 (40%)		13 (43%)		
Lactoferrin (μg L^{-1})	146·5 ± 54·3	241·0 ± 76·7	< 0·001	219·4 ± 68·1	< 0·001	262·6 ± 79·9	< 0·001	< 0·05
Elastase (μg L^{-1})	54·8 ± 16·3	155·5 ± 69·7	< 0·001	105·2 ± 20·9	< 0·001	205·9 ± 64·9	< 0·001	< 0·001

AP, active psoriasis; IP, inactive psoriasis; BMI, body mass index; NS, nonsignificant.

Table 3. Erythrocyte antioxidant defences, plasma lipid peroxidation and antioxidant capacity (mean ± SD) for controls and psoriasis patients

	Controls ($n = 30$)	IP + AP ($n = 60$)	*P*-value (IP + AP)/controls	IP ($n = 30$)	*P*-value IP/controls	AP ($n = 30$)	*P*-value AP/controls	*P*-value IP/AP
Age (years)	45·2 ± 14·6	45·6 ± 12·0	NS	45·7 ± 12·8	NS	45·6 ± 11·4	NS	NS
BMI (kg m^{-2})	24·1 ± 1·8	24·8 ± 3·1	NS	24·4 ± 3·5	NS	25·2 ± 2·5	NS	NS
Smokers	13 (43%)	25 (42%)		12 (40%)		13 (43%)		
Vitamin E (nmol g^{-1} Hb)	13·2 ± 3·1	11·9 ± 2·5	< 0·05	12·3 ± 2·6	NS	11·4 ± 2·4	< 0·05	NS
Catalase (IU $\times 10^4$ g^{-1} Hb)	5·77 ± 1·26	5·45 ± 1·22	NS	5·55 ± 1·29	NS	5·35 ± 1·16	NS	NS
GPx (IU g^{-1} Hb)	59·0 ± 11·6	50·9 ± 11·1	< 0·01	51·7 ± 10·1	< 0·05	50·1 ± 12·2	< 0·01	NS
SOD (IU g^{-1} Hb)	1495 ± 222	1587 ± 249	NS	1561 ± 247	NS	1612 ± 252	NS	NS
GSH (μmol g^{-1} Hb)	11·75 ± 1·03	9·78 ± 1·09	< 0·001	9·89 ± 1·09	< 0·001	9·66 ± 1·09	< 0·001	NS
Se (μg L^{-1})	59·9 ± 15·2	52·9 ± 13·9	< 0·05	54·8 ± 12·9	NS	50·7 ± 15·0	< 0·05	NS
TAS (mmol L^{-1})	1·63 ± 0·18	1·38 ± 0·25	< 0·001	1·51 ± 0·16	< 0·05	1·25 ± 0·25	< 0·001	< 0·001
TBA ($\times 10^{-3}$ mmol L^{-1})	2·13 ± 0·52	5·89 ± 1·30	< 0·001	5·06 ± 1·10	< 0·001	6·72 ± 0·89	< 0·001	< 0·001
TBA/TAS	1·32 ± 0·38	4·56 ± 1·85	< 0·001	3·40 ± 0·92	< 0·001	5·72 ± 1·82	< 0·001	< 0·001

IP, inactive psoriasis; AP, active psoriasis; BMI, body mass index; Hb, haemoglobin; GPx, glutathione peroxidase; SOD, superoxide dismutase; GSH, reduced glutathione; Se, selenium; TAS, total plasma antioxidant capacity; TBA, thiobarbituric acid reactivity, a measure of plasma lipid peroxidation; NS, nonsignificant.

found for GPx activity ($P < 0·05$) and for GSH ($P < 0·001$). Patients with severe psoriasis presented an enhancement in these changes. We observed a significant reduction in membrane vitamin E ($P < 0·05$), GPx activity ($P < 0·01$), Se ($P < 0·05$) and GSH ($P < 0·001$).

Although a reduced RBC antioxidant capacity was observed in AP patients, no significant differences were observed with worsening of the disease (IP vs. AP).

A reduction in total plasma antioxidant capacity was also observed in psoriasis patients (Table 3). We found a significantly reduced value for TAS ($P < 0·001$) and

higher values for TBA ($P < 0{\cdot}001$) and for the ratio TBA/TAS ($P < 0{\cdot}001$). The same changes were observed for mild psoriasis, when compared with controls, and changes were further enhanced in severe psoriasis. When comparing these values according to the severity of the disease (IP vs. AP) we found significant differences, denoting a linkage of severe psoriasis with a reduction in the erythrocyte antioxidant capacity and the development of oxidative stress within the cell.

Table 4 shows the erythrocyte membrane damage observed in controls and in psoriasis patients. When compared with the control group, we found that total psoriasis patients showed higher membrane lipid peroxidation and a higher susceptibility to membrane lipid peroxidation (both with TBA and with PnA assay) ($P < 0{\cdot}001$). Moreover, we found in psoriasis patients a significantly lower membrane fluidity, as shown by the polarization probes DPH and TMA-DPH ($P < 0{\cdot}001$) in association with a higher Chol/Phlip ratio ($P < 0{\cdot}01$). MBH, a marker of oxidative stress in RBCs, also showed a significantly higher value ($P < 0{\cdot}001$). The band 3 profile presented a significantly different pattern for psoriasis patients: the percentage of HMWAg was almost double the control value and the band 3 monomer decreased ($P < 0{\cdot}001$); no significant difference was found for Pfrag of band 3. The same modifications were observed in IP and AP patients when compared with controls, except for the band 3 profile of AP patients, in which the reduction in Pfrag reached statistical significance. All these RBC membrane modifications were enhanced in AP patients, and when we compared IP vs. AP we found significantly different values with the exception of Chol/Phlip ratio and band 3 profile.

To provide further evidence of the involvement of leucocytes with plasma and RBC damage, we looked for significant correlations between them. We found a positive correlation of both total WBC and neutrophils with the levels of lipid peroxidation in plasma ($R = 0{\cdot}548$, $P < 0{\cdot}001$; $R = 0{\cdot}455$, $P < 0{\cdot}001$, respectively). A positive correlation was also found for elastase with both total WBC and neutrophils ($R = 0{\cdot}617$, $P < 0{\cdot}001$; $R = 0{\cdot}799$, $P < 0{\cdot}001$), and for lactoferrin with neutrophil count ($R = 0{\cdot}515$, $P < 0{\cdot}001$). Stronger correlations were found in severe psoriasis ($R = 0{\cdot}589$, $P < 0{\cdot}001$; $R = 0{\cdot}848$, $P < 0{\cdot}001$; $R = 0{\cdot}642$, $P < 0{\cdot}001$, respectively). The levels of lipid peroxidation in the RBC membrane were negatively correlated with the enzymatic activity of GPx and Cat ($R = -0{\cdot}506$, $P < 0{\cdot}001$; $R = -0{\cdot}397$, $P < 0{\cdot}001$, respectively), and these values were higher for severe psoriasis ($R = -0{\cdot}526$, $P < 0{\cdot}001$; $R = -0{\cdot}643$, $P < 0{\cdot}001$, respectively). We also observed a positive correlation between the levels of lipid peroxidation in plasma and in the RBC membrane ($R = 0{\cdot}591$, $P < 0{\cdot}001$). Again, a positive correlation was observed between membrane lipid peroxidation and the band 3 aggregation ($R = 0{\cdot}525$, $P < 0{\cdot}001$). Negative correlations were found in severe psoriasis between the aggregation of band 3 and the activity of Cat and GPx, and the GSH levels ($R = -0{\cdot}467$,

Table 4. Erythrocyte membrane damage (mean ± SD) for controls and psoriasis patients

	Controls ($n = 30$)	IP + AP ($n = 60$)	*P*-value (IP + AP)/controls	IP ($n = 30$)	*P*-value IP/controls	AP ($n = 30$)	*P*-value AP/controls	*P*-value IP/AP
DPH	0·332 ± 0·006	0·358 ± 0·007	< 0·001	0·354 ± 0·004	< 0·001	0·362 ± 0·007	< 0·001	< 0·001
TMA-DPH	0·382 ± 0·006	0·412 ± 0·007	< 0·001	0·410 ± 0·006	< 0·001	0·414 ± 0·006	< 0·001	< 0·05
Chol/Phlip	0·748 ± 0·137	0·830 ± 0·104	< 0·01	0·809 ± 0·090	< 0·05	0·852 ± 0·114	< 0·01	NS
TBA (0 h)	0·162 ± 0·019	0·234 ± 0·066	< 0·001	0·209 ± 0·055	< 0·001	0·259 ± 0·067	< 0·001	< 0·01
TBA (2 h)	0·351 ± 0·037	0·488 ± 0·142	< 0·001	0·437 ± 0·132	< 0·01	0·539 ± 0·134	< 0·001	< 0·01
PnA	− 2·63 ± 1·01 ($n = 29$)	− 5·08 ± 1·70 ($n = 34$)	< 0·001	− 4·55 ± 1·41 ($n = 18$)	< 0·001	− 5·62 ± 1·82 ($n = 17$)	< 0·001	< 0·05
Band 3 HMWAg (%)	9·7 ± 5·2	17·8 ± 4·8	< 0·001	16·7 ± 4·4	< 0·001	18·9 ± 5·0	< 0·001	NS
Band 3 (%)	63·5 ± 4·2	56·7 ± 4·8	< 0·001	56·2 ± 3·2	< 0·001	57·2 ± 6·0	< 0·001	NS
Pfrag (%)	26·8 ± 4·5	25·5 ± 5·9	NS	27·2 ± 6·6	NS	23·9 ± 4·6	< 0·05	NS
MBH ($\times 10^{-4}$%)	96 ± 32	257 ± 134	< 0·001	202 ± 109	< 0·01	312 ± 137	< 0·001	< 0·05

IP, inactive psoriasis; AP, active psoriasis; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene (values show fluorescence of polarization); Chol, cholesterol; Phlip, phospholipids; TBA, thiobarbituric acid (μmol g^{-1} haemoglobin); PnA, parinaric acid (slope value); HMWAg, high molecular weight aggregates; Pfrag, proteolytic fragments; MBH, membrane-bound haemoglobin; NS, nonsignificant.

Table 5. Lipid profile (mean ± SD) for controls and psoriasis patients

	Controls ($n = 40$)	IP + AP ($n = 60$)	*P*-value (IP + AP)/controls	IP ($n = 30$)	*P*-value IP/controls	AP ($n = 30$)	*P*-value AP/controls	*P*-value IP/AP
Age (years)	47·4 ± 13·3	45·4 ± 11·6	NS	45·1 ± 12·0	NS	45·6 ± 11·4	NS	NS
BMI (kg m^{-2})	24·4 ± 1·8	24·9 ± 3·0	NS	24·5 ± 3·4	NS	25·2 ± 2·5	NS	NS
Smokers	17 (42·5%)	24 (40%)		11 (37%)		13 (43%)		
Chol (mg dL^{-1})	199·8 ± 27·7	219·6 ± 39·4	< 0·01	207·7 ± 41·7	NS	231·4 ± 33·7	< 0·001	< 0·05
TG (mg dL^{-1})	92·6 ± 27·3	119·6 ± 54·2	< 0·01	99·2 ± 52·4	NS	140·0 ± 48·7	< 0·001	< 0·01
HDLc (mg dL^{-1})	50·0 ± 3·2	44·7 ± 5·7	< 0·001	45·9 ± 5·7	< 0·01	43·5 ± 5·5	< 0·001	NS
LDLc (mg dL^{-1})	131·3 ± 27·9	151·0 ± 37·6	< 0·01	142·0 ± 39·9	NS	159·9 ± 33·4	< 0·001	NS
VLDLc (mg dL^{-1})	18·6 ± 6·3	24·3 ± 10·7	< 0·01	20·5 ± 10·5	NS	28·0 ± 9·7	< 0·001	< 0·01
Phlip (mg dL^{-1})	200·9 ± 28·1	200·4 ± 32·6	NS	196·9 ± 28·6	NS	203·9 ± 36·2	NS	NS
Chol/Phlip	1·01 ± 0·19	1·12 ± 0·25	< 0·05	1·07 ± 0·22	NS	1·17 ± 0·26	< 0·01	NS

IP, inactive prosiasis; AP, active psoriasis; BMI, body mass index; Chol, cholesterol; TG, triglycerides; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; VLDLc, very low-density lipoprotein cholesterol; Phlip, phospholipids, NS, nonsignificant.

$P < 0·001$; $R = -0·501$, $P < 0·001$; $R = -0·558$, $P < 0·001$, respectively).

Table 5 shows the lipid profile presented by controls and psoriasis patients. When compared with controls, we found that total psoriasis patients presented significantly higher values for Chol ($P < 0·01$), TG ($P < 0·01$), LDLc ($P < 0·01$), VLDLc ($P < 0·01$) and Chol/Phlip ratio ($P < 0·05$); significantly lower values were found for HDLc ($P < 0·001$). Patients with mild psoriasis presented only a significant reduction in HDLc ($P < 0·01$), while AP patients presented the same significant changes as for total patients. When comparing IP vs. AP patients a significant rise was found for Chol ($P < 0·05$), TG ($P < 0·01$) and VLDLc ($P < 0·01$).

Discussion

Psoriasis as a clinically inflammatory skin disease seems to impose an oxidative stress condition.[1,2] The activation of the inflammatory cells in psoriasis may result in increased leucocyte activation products in the peripheral blood,[3,4] which may induce oxidative and proteolytic modifications to plasma constituents and neighbouring cells such as circulating erythrocytes.[10–12] Considering that the RBC has a very limited biosynthesis capacity, physical and/or chemical damage will accumulate throughout its life span. Therefore, we considered it reasonable that the erythrocyte could provide a cumulative marker of oxidative and proteolytic stress in psoriasis, and also a marker of worsening of the disease. In this perspective, we studied the erythrocyte in psoriasis patients, half with mild psoriasis and the other half with severe psoriasis. The RBC was evaluated in different ways, by searching for oxidative and/or proteolytic damage, and by searching for a correlation with leucocyte activation and oxidative stress development.

We found that psoriasis patients presented a higher number of WBCs (Table 2) and that this rise was mainly due to an increased number of neutrophils. The higher plasma levels of neutrophil activation products—elastase and lactoferrin—in psoriasis patients may reflect the higher number of neutrophils and/or higher neutrophil activation. It is known that neutrophil activation is also linked to the production of oxygen metabolites, which may trigger oxidative modifications in plasma constituents and in cell membranes.[5–12] The evaluation of plasma levels of lipid peroxidation (Table 3) showed a significantly higher value in psoriasis, along with a significantly reduced total plasma antioxidant capacity. The TBA/TAS ratio clearly reveals the development of an oxidative stress condition in psoriasis.

Some oxygen metabolites are highly reactive, whereas others, such as hydrogen peroxide, are less reactive and have the ability to diffuse easily across the cell membranes, namely the erythrocyte membranes. They may therefore impose oxidative modifications upon the membrane and within the RBC. Gaining the

inside of the RBC, they will trigger the erythrocyte antioxidant defence mechanisms. When these RBC defences are depleted, an oxidative stress will develop within the cell and therefore oxidative modifications will appear. In psoriasis patients we observed a reduction in the erythrocyte antioxidant defences. We found reduced levels of GSH and Se, and reduced enzymatic activity for Cat and GPx (Table 3). These enzymes are particularly involved in the detoxification of the cell in oxygen radicals and it seems that the failure of this mechanism may account for the lipid peroxidation of the RBC membrane, as suggested by the significant negative correlations of Cat and GPx activity with RBC membrane lipid peroxidation. Moreover, the RBC membrane levels of the antioxidant vitamin E were also reduced in psoriasis patients.

Together, the above results suggest the development of oxidative stress within the RBC, with failure of membrane and cytoplasmic antioxidant defences (Table 3). Strengthening these observations, we found (Table 4) that the RBC membrane lipid peroxidation and the membrane susceptibility to lipid peroxidation were higher in psoriasis patients, as shown by the higher levels of TBA in RBC membranes, before and after 2 h of *in vitro* imposed oxidative stress. The higher value of membrane lipid peroxidation in patients was corroborated by using the PnA assay. These results, and those reflecting a depletion in membrane and cytoplasmic antioxidant defences, are mostly in agreement with previous observations of other authors.[20,21,49–51] In the case of SOD, some authors reported a reduced activity in psoriasis patients, while others, including ourselves, found an increase.

In searching for oxidative modifications which would result from the developed oxidative stress, the evaluation of MBH, denoting the oxidation of Hb, showed a sharp rise in psoriasis patients. It is known that the linkage of denatured Hb to the RBC membrane at the cytoplasmic pole of the protein band 3 favours the aggregation of this transmembrane protein.[18] In accordance with this, we found a different band 3 profile in psoriasis patients, which showed a significant rise in HMWAg of band 3 (Table 4). The development and accumulation of the several oxidative modifications in the RBC must lead to changes in the osmotic fragility of the cell and in the membrane fluidity. We found an increased osmotic fragility (Table 1) and a reduced fluidity in the erythrocytes from psoriasis patients (Table 4). We observed an increased membrane fluorescence polarization, when using DPH and TMA-DPH as probes, and a rise in the Chol/Phlip ratio, both reflecting a reduction in membrane fluidity. The higher RBC membrane Chol/Phlip ratio in psoriasis patients was linked to a higher serum Chol/Phlip ratio (Table 5). Thus, the observed changes in membrane fluidity may reflect the dyslipidaemia and the enhanced production of ROS, which increases lipid peroxidation and induces depletion of the antioxidant defences. Psoriasis has been associated with oxidative stress, abnormal plasma lipid metabolism and a high frequency of cardiovascular events.[1,52] The changes we observed in the lipid profile of psoriasis patients were all risk changes and seemed to be enhanced in severe psoriasis.

Reductions in total RBC count, MCV and enzymatic activity with failure of the cytoplasmic and membrane antioxidant defences, and a rise in the osmotic fragility, are all marks of RBC senescence or damage, and these changes were observed in psoriasis patients (Table 1). Plasma levels of total bilirubin were higher in psoriasis patients, denoting an enhanced RBC damaging and/or ageing process and the consequent enhancement in RBC removal. Strengthening this hypothesis, and expressing the physiological mechanism to counterbalance a higher RBC removal, we found a higher number of reticulocytes in psoriasis patients.

It is known that the rise in the levels of some cytokines along inflammatory processes may underlie a reduction in erythropoietin production, in iron release from reticuloendothelial stores, in the proliferation of erythroid progenitors, and therefore in the number of circulating RBCs.[53] In the present study we found a reduction in RBC count; however, it was accompanied by changes denoting an enhancement in RBC damage, followed by changes denoting a slightly enhanced RBC removal and a slightly enhanced production of RBCs, as shown by a higher reticulocyte count. Therefore, considering that subjects with deficiencies in erythropoietic nutrients were excluded from the study, it seems that the reduction in RBC count is mainly connected with the development of oxidative stress and worsening of psoriasis.

We looked for a correlation between total WBC and neutrophil counts and plasma lipid peroxidation to clarify the role played by those cells in this disease, and found a significant positive correlation between them. This suggests that the leucocyte rise is linked to cell activation, as the activated neutrophils are important sources of oxygen metabolites, which probably accounted for the rise in plasma lipid peroxidation. We also found a positive correlation between neutrophil count and the plasma levels of the neutrophil activation

products elastase and lactoferrin, reflecting the rise and/or activation of neutrophils.

The comparison of mild (IP) and severe (AP) psoriasis with the controls showed that most of the changes seen in psoriasis patients (IP + AP) were already observed in mild psoriasis, with the exception of MCH, total reticulocytes, WBCs and lymphocytes, RBC membrane vitamin E and selenium. In severe psoriasis all the changes became significantly different from the controls, suggesting that the worsening of the disease is associated with the enhancement of neutrophil activation, and therefore with an even more accelerated RBC damage and/or ageing process and a higher RBC removal and reticulocyte release.

Our study focused on the erythrocyte as a marker for the worsening of psoriasis, which suddenly and unexpectedly develops from mild to severe with enlargement of the psoriasis lesions. Therefore, looking for an early predictive index for the development of a psoriasis crisis, we compared the values obtained in mild and severe psoriasis. We found that the enlargement of psoriasis lesions was associated with a reduction in RBCs, a rise in reticulocytes, and the development of erythrocyte changes denoting enhanced damage, namely a rise in osmotic fragility, in membrane lipid peroxidation and in its susceptibility to lipid peroxidation, and in MBH, showing a failure in the cytoplasmic antioxidant defences. The worsening of the disease was also associated with a reduction in RBC membrane fluidity. Considering that, as suggested by the present study, erythrocyte damage is enhanced in psoriasis, particularly in severe psoriasis, these patients are more prone to develop anaemia. This is probably of particular value for those patients with severe psoriasis, presenting excessive losses of iron in scales, and in those who, being resistant to conventional therapeutic agents, are under methotrexate therapy.[54]

Neutrophil count and its activation products elastase and lactoferrin, as well as the oxidative damage of the RBC membrane, may provide predictive markers for worsening of psoriasis. Values for elastase and MBH were almost double in severe psoriasis. Therefore, we suggest that a regular evaluation of these potential markers in patients with mild psoriasis could be of value in predicting in advance the worsening of the disease and therefore enabling earlier therapeutic intervention. Moreover, our data clearly suggest the need for a therapeutic study on the value of inhibiting the neutrophil activation process and its deleterious effects upon neighbouring cells.

In a previous study,[1] we found that psoriasis patients presented a different lipid profile, with several modifications of risk for cardiovascular events, and that these changes were enhanced with worsening of the disease. The activation of leucocytes resulting in increased levels of leucocyte activation products in the peripheral blood of psoriasis patients may favour atherogenesis by promoting the oxidation of LDL. Moreover, these activation products seem to contribute to a higher erythrocyte membrane rigidity by imposing oxidative modifications to RBCs. The rise in circulating WBCs, with a reduced capacity to deform, and the rise in RBCs with a reduced membrane elasticity, may enhance the inflammatory and the RBC damage process, by increasing the blood viscosity and reducing the blood flow at the psoriasis lesions. In addition, we wonder if the increased blood viscosity, resulting from the association of leucocyte activation and RBC damage with dyslipidaemia and a reduced antioxidant capacity in psoriasis, could not explain in part the higher prevalence of cardiovascular events in these patients.

In summary, our data show a reduced number of erythrocytes in psoriasis patients, and present several changes denoting an enhanced damage and/or ageing process, which seem to be strongly connected with neutrophil activation, oxidative stress and worsening of psoriasis.

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