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Clinical and Laboratory Investigations

The inflammatory response in mild and in severe psoriasis

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Summary

Background Psoriasis is a chronic and recurrent inflammatory skin disease. The inflammatory response represents a fundamental ability of the organism to protect itself from infectious agents and from injury.

Objectives To evaluate the inflammatory response in mild and in severe psoriasis, to evaluate the endogenous systems counterbalancing the deleterious effects of the inflammation products, and to establish values of prognostic significance.

Methods The study was performed in a control group ($n = 40$) and in 60 patients with psoriasis vulgaris, half presenting with mild psoriasis, and the other half with severe psoriasis. We evaluated total and differential leucocyte count; elastase, lactoferrin and lipid peroxidation as markers of neutrophil activation; total plasma antioxidant capacity (TAS), transferrin, ceruloplasmin, α_1-antitrypsin and α_2-macroglobulin as markers of the endogenous antioxidant and antiprotease systems; and fibrinogen, erythrocyte sedimentation rate, C-reactive protein (CRP), haptoglobin, C3 and C4 complement proteins as markers of inflammation.

Results Our data suggested that psoriasis is an inflammatory condition in which neutrophils seem to play a crucial role by contributing to the development of oxidative and proteolytic stress. The worsening of the disease seemed to be linked to the enhancement of the inflammatory response and of the imbalance between neutrophil activation products and their inhibitors.

Conclusions We propose values for elastase, CRP, elastase/α_2-macroglobulin, elastase/α_1-antitrypsin, thiobarbituric acid/TAS and elastase/neutrophil ratios with prognostic significance for the worsening of psoriasis.

Key words: C-reactive protein, inflammation, neutrophil activation, neutrophilic elastase, oxidative stress, psoriasis activity

The inflammatory response represents a fundamental ability of the organism to protect itself against exposure to infectious agents and to injury.[1] It occurs in vascular tissue and involves complex interactions between blood cells, plasma mediator systems and the microvasculature. An inflammatory response usually includes local haemodynamic changes, alterations in microvascular permeability and a series of cellular events leading to accumulation of leucocytes and to their activation.

Psoriasis is known as a chronic and recurrent inflammatory skin disease, and its worsening has been linked with oxidative stress.[2–4] As any inflammatory disease, psoriasis often presents a rise in white blood cells (WBCs), namely in neutrophils. Clinically active psoriasis lesions show infiltration of WBCs, mainly of neutrophils, and several studies report high levels of neutrophil activation products in psoriatic lesions[5,6]

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and in the peripheral blood of these patients.[7] The activation of neutrophils triggers a set of functional and metabolic responses, including degranulation, enzyme release and generation of reactive oxygen species (ROS).[8]

ROS have been shown to mediate inflammatory processes and to be involved in oxidative reactions such as lipid peroxidation and protein oxidation.[9,10] They may greatly amplify the inflammatory response, but they may also contribute to tissue damage. To counterbalance the destructive effects of these oxidants there are endogenous antioxidant systems, such as the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, promoting the detoxification of ROS;[11] the proteins ceruloplasmin and transferrin, by linking iron, avoid the development of the Fenton reaction. This reaction appears to be an important mechanism for generation of the hydroxyl radical, the more deleterious oxygen metabolite, from hydrogen peroxide in the presence of free iron and reducing agents, namely superoxide, ascorbate and lactate.[8,12]

The degranulation of activated neutrophils appears to be important in the inflammatory response and in tissue damage. For instance, lactoferrin released by the specific neutrophil granules[13] seems to promote neutrophil–endothelial cell adhesion and, as a source of iron, it may also promote the Fenton reaction. Neutral proteases released by the azurophilic granules of the activated neutrophils,[13] such as elastase, mediate tissue damage by degradation of matrix proteins. Again, to limit the deleterious effects of these granular proteases there are endogenous antiprotease systems.[14,15] The most important antiproteases are α_1-antitrypsin and α_2-macroglobulin, which are both synthesized and secreted in larger quantity when triggered by an inflammatory process.

The aim of this study was to evaluate the extent of the inflammatory response in mild and severe psoriasis, and to evaluate the balance between the products of the inflammatory response and the endogenous systems counterbalancing their deleterious effects. We believe that if the balance between the two systems is broken, it would lead to enhanced tissue damage and inflammation, with worsening of psoriasis. Moreover, by comparing the inflammatory response in mild and severe psoriasis, we will search for markers of worsening of the disease.

We studied a control group and a group of patients with psoriasis vulgaris, half presenting with mild psoriasis and the other half with severe psoriasis. Besides total and differential WBC count, we evaluated plasma levels of elastase and lactoferrin, as traducers of WBC degranulation and activation. As this activation is also linked to the generation of ROS, we evaluated plasma lipid peroxidation as an indirect marker of their production. To study the capacity of the endogenous antioxidant systems, total plasma antioxidant capacity, as well as plasma levels of ceruloplasmin and transferrin, were evaluated. The endogenous antiprotease system was evaluated by measuring the plasma levels of α_1-antitrypsin and α_2-macroglobulin. Fibrinogen, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), haptoglobin, and C3 and C4 complement proteins were evaluated as markers of inflammation.

Materials and methods

Subjects

The protocol used was approved by the Committee on Ethics of the University Hospital of Coimbra and all the patients and controls gave informed consent. As controls, we studied 40 apparently healthy adults [55% men and 45% women, mean ± SD age 47 ± 13 years, body mass index (BMI) 24·4 ± 1·8], with no history of any skin disease, and presenting with normal haematological and biochemical values. The selected patients consisted of 60 adults (56% men and 43% women, mean ± SD age 46 ± 12 years, BMI 24·7 ± 3·1), 30 of them presenting with mild psoriasis vulgaris (53% men and 46% women, mean ± SD age 47 ± 13 years, BMI 24·2 ± 3·6), and the other 30 presenting with severe psoriasis vulgaris (60% men and 40% women, mean ± SD age 46 ± 11 years, BMI 25·2 ± 2·5). The disease was diagnosed from 0·5 to 50 years before this study.

We performed a clinical study and blood analysis of the patients and controls. Individuals presenting deficiencies in erythropoietic nutrients or with other associated diseases, namely diabetes mellitus, cardiovascular, liver or kidney diseases, were excluded from the study. Individuals with other skin diseases and alcoholics were also excluded.

Psoriasis was graded according to the Psoriasis Area and Severity Index (PASI) presenting at the time of blood collection.[16] Half of the patients had severe psoriasis or active psoriasis (AP; PASI > 3), and the other half had mild psoriasis or inactive psoriasis (IP; PASI < 3).

To assess the changes imposed by psoriasis *per se*, 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 anti-inflammatories.

Collection and preparation of blood samples

Blood was collected from the subjects, fasted for 12 h, with and without anticoagulant, in order to obtain whole blood, plasma and serum. Sodium citrate and ethylenediamine tetraacetic acid (EDTA) were used as anticoagulants. The first was used to collect blood samples for the evaluation of plasma fibrinogen and ESR. For the other evaluations in plasma and in whole blood, EDTA was used as anticoagulant. None of the samples was icteric or haemolysed.

Assays

Total and differential leucocyte count. Whole blood was used for these evaluations. An automatic blood cell counter was used (Autocounter AC 970) to measure the total and differential WBC count.

Oxidative stress. As an indirect marker of oxygen metabolite production we evaluated plasma lipid peroxidation by measuring the thiobarbituric acid (TBA) reactivity (TBA assay).[17] Total plasma antioxidant capacity was evaluated by a colorimetric assay (TAS; Randox Laboratories, Crumlin, U.K.).

Serum levels of transferrin and ceruloplasmin were evaluated by nephelometry. The levels of the immunocomplexes formed with the specific antibodies (N Antiserum to human transferrin, ceruloplasmin and haptoglobin; Dade Behring) were detected by using a nephelometer (BN II; Dade Behring, Marburg, Germany).

Products of neutrophil degranulation. Plasma concentrations of polymorphonuclear elastase and lactoferrin were evaluated by enzyme immunoassays (PMN Elastase IMAC immunoassay; Merck, Darmstadt, Germany; Bioxytech lactof enzyme immunoassay, Oxis International, Portland, O.R., U.S.A., respectively).

Markers of inflammation. To measure ESR according to the Westergren method,[18] whole blood was used.

Serum CRP and haptoglobin were evaluated by nephelometry (N High sensitivity CRP; N Antiserum to human transferrin, ceruloplasmin and haptoglobin; Dade Behring).

To evaluate the plasma levels of fibrinogen we used a turbidimetric assay (Fibrinogen 'O'; DiaMed, Morat, Switzerland).

The serum levels of complement proteins C3 and C4 were evaluated by nephelometry (Antiserum to human complement factor C3 and C4; Dade Behring).

Endogenous antiproteases. The plasma levels of the endogenous antiproteases α_1-antitrypsin and α_2-macroglobulin were evaluated by nephelometry (N Antiserum to human α_1-antitrypsin and α_2-macroglobulin; Dade Behring).

Statistical analysis

The statistical analysis was performed using the SPSS package (SPSS, Chicago, IL, U.S.A.). To evaluate the 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 nongaussian distribution, as was the case for elastase and CRP. $P < 0{\cdot}05$ was considered statistically significant. Measurements are expressed as mean ± SD. The strength of the association between the parameters was estimated by the Pearson correlation coefficient. To draw the graphs we used Microsoft Excel software.

Results

We analysed the results to study the differences between controls and patients, between mild and severe psoriasis (IP vs. AP), and to find values of prognostic significance for worsening of the disease.

We found a significantly higher WBC count in patients ($P < 0{\cdot}001$) (Table 1), which was mainly due to increased neutrophils ($P < 0{\cdot}001$). A rise in WBC count was also found in IP and AP, although this was significant only in AP ($P < 0{\cdot}001$). Again, in both groups this rise was mainly due to neutrophils ($P < 0{\cdot}001$). Comparing IP vs. AP patients (Table 1), we found for AP significantly higher total ($P < 0{\cdot}001$) and differential WBC count. Figure 1A shows that only nine (30%) AP patients presented a WBC count higher than controls ($> 9{\cdot}21 \times 10^9\ L^{-1}$). However, four (13%) IP (Fig. 1B) and 19 (63%) AP patients showed a neutrophil count higher than controls ($> 5{\cdot}43 \times 10^9\ L^{-1}$).

The inflammatory response study is shown in Table 2. We found significant differences for all parameters in patients ($P < 0{\cdot}05$ for ceruloplasmin and transferrin; $P < 0{\cdot}001$ for all others). In IP we found the same changes, all being significant ($P < 0{\cdot}001$) excepting ceruloplasmin, transferrin and TAS. In AP the enhanced changes reached significantly higher values, excluding TAS, which was significantly lower. Comparing IP vs. AP, we observed a significant enhancement in all parameters, excepting ceruloplasmin, transferrin and complement protein C3 (Table 2).

Table 1. Total and differential white blood cell (WBC) count (mean ± SD) for controls and psoriasis patients

	C ($n = 40$)	IP + AP ($n = 60$)	*P*-value (IP + AP)/C	IP ($n = 30$)	*P*-value IP/C	AP ($n = 30$)	*P*-value AP/C	*P*-value IP/AP
WBC	6·69 ± 1·30	7·72 ± 1·52	< 0·001	6·99 ± 1·31	NS	8·46 ± 1·37	< 0·001	< 0·001
Neutrophils	3·93 ± 0·77	5·23 ± 1·03	< 0·001	4·66 ± 0·83	< 0·001	5·81 ± 0·89	< 0·001	< 0·001
Lymphocytes	2·32 ± 0·45	2·58 ± 0·49	< 0·01	2·39 ± 0·44	NS	2·78 ± 0·47	< 0·001	< 0·01
Monocytes	0·24 ± 0·05	0·21 ± 0·05	< 0·01	0·19 ± 0·03	< 0·001	0·23 ± 0·05	NS	< 0·01

WBCs, neutrophils, lymphocytes, monocytes ($\times 10^9$ L^{-1}); C, control; IP, inactive psoriasis; AP, active psoriasis; NS, not significant.

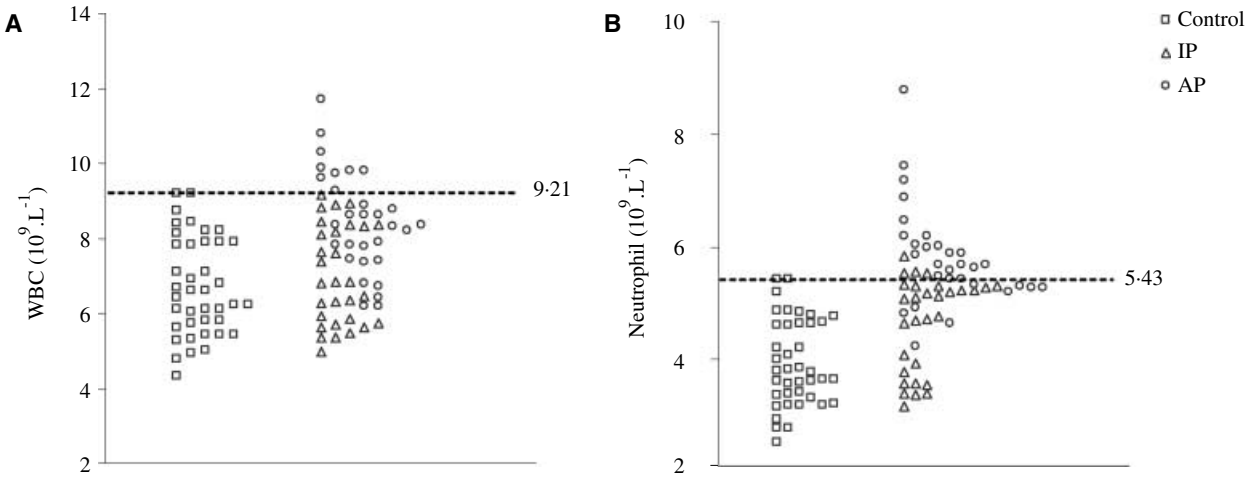

Figure 1. Total white blood cell (WBC) count (A) and neutrophil count (B) in the 40 controls (□), in the 30 patients with inactive psoriasis (IP, △) and in the 30 patients with active psoriasis (AP, ○). The control values are under the line (- - -).

Concerning WBC activation products, Figure 2A shows that 23 (77%) AP and 14 (47%) IP patients presented values of lactoferrin higher than controls (> 240·1 μg L^{-1}). For elastase (Fig. 2B), a value higher than in controls (> 82·4 μg L^{-1}) was observed in 57 (95%) patients. Considering only patients, 21 (70%) AP patients presented values higher than IP patients (> 142·3 μg L^{-1}). The TBA evaluation, an indirect marker of ROS generation, showed (Fig. 2C) that all patients presented lipid peroxidation values higher than controls (> 2·92 μmol L^{-1}).

Some inflammatory markers showed noteworthy differences (Fig. 3). In 29 (43%) patients we found fibrinogen levels higher than in controls (> 339·0 mg dL^{-1}) (Fig. 3A), 21 (70%) of them being AP patients. In 32 (53%) patients, the ESR (Fig. 3B) presented higher values than controls (> 23·0 mm in the first hour), 25 (80%) of them being AP patients. For haptoglobin (Fig. 3C), we found that 58 patients (97%) presented values higher than in controls (> 188·7 mg dL^{-1}). The most striking differences found between controls, IP and AP patients were in CRP (Fig. 3D). The 30 IP patients (100%) presented values higher than controls (> 0·36 mg L^{-1}) and values lower than those shown by the 30 AP patients (100%) (< 1·04 mg L^{-1}).

Concerning the inhibitors of WBC activation products (Fig. 4), we found that 15 AP patients (50%) and one IP patient presented a TAS value lower than in controls (< 1·27 mmol L^{-1}) (Fig. 4A). For α_1-antitrypsin (Fig. 4B), we found that 40 patients (67%) presented a value higher than in controls (> 163·4 mg dL^{-1}), 24 (60%) of these being AP patients. For α_2-macroglobulin (Fig. 4C), 39 patients (65%) presented a value higher than in controls (> 228·4 mg dL^{-1}), 26 (87%) of these being AP patients.

The ratios between elastase and its inhibitors (Table 3) showed a significant imbalance in patients in general ($P < 0·001$), as well as in IP and AP patients separately ($P < 0·001$), when compared with controls. IP vs. AP showed a significant rise in these ratios ($P < 0·001$). The individual results (Fig. 5) showed that 36 patients (60%) presented an elastase/α_2-macroglobulin ratio higher than in controls (> 0·50)

Table 2. Inflammatory markers, products of neutrophil activation and of their inhibitors (mean ± SD) for controls and psoriasis patients

	C ($n = 40$)	IP + AP ($n = 60$)	*P*-value (IP + AP)/C	IP ($n = 30$)	*P*-value IP/C	AP ($n = 30$)	*P*-value AP/C	*P*-value IP/AP
Lactoferrin	146·4 ± 54·3	241·0 ± 76·7	< 0·001	219·4 ± 68·0	< 0·001	262·6 ± 79·9	< 0·001	< 0·05
TBA	1·86 ± 0·41	5·97 ± 1·23	< 0·001	5·23 ± 1·07	< 0·001	6·72 ± 0·89	< 0·001	< 0·001
Ceruloplasmin	42·4 ± 10·7	47·2 ± 9·1	< 0·05	45·2 ± 9·2	NS	49·2 ± 8·7	< 0·01	NS
Transferrin	204·0 ± 24·7	215·6 ± 20·3	< 0·05	212·8 ± 15·1	NS	218·5 ± 24·4	< 0·05	NS
TAS	1·60 ± 0·18	1·39 ± 0·27	< 0·001	1·54 ± 0·20	NS	1·25 ± 0·25	< 0·001	< 0·001
Elastase	54·8 ± 16·3	155·5 ± 69·7	< 0·001	105·2 ± 20·9	< 0·001	205·9 ± 64·9	< 0·001	< 0·001
α_1-antitrypsin	139·8 ± 17·0	170·0 ± 20·2	< 0·001	162·3 ± 19·3	< 0·001	177·8 ± 18·4	< 0·001	< 0·01
α_2-macroglobulin	186·1 ± 27·1	242·1 ± 38·1	< 0·001	220·1 ± 34·4	< 0·001	264·2 ± 27·6	< 0·001	< 0·001
Fibrinogen	290·6 ± 30·5	344·7 ± 55·5	< 0·001	321·7 ± 36·2	< 0·001	367·6 ± 62·2	< 0·001	< 0·01
ESR	9·6 ± 6·2	24·3 ± 9·4	< 0·001	18·3 ± 7·5	< 0·001	30·2 ± 7·1	< 0·001	< 0·001
CRP	0·31 ± 0·02	0·90 ± 0·27	< 0·001	0·63 ± 0·03	< 0·001	1·16 ± 0·07	< 0·001	< 0·001
Haptoglobin	137·0 ± 17·7	224·2 ± 22·2	< 0·001	210·6 ± 17·5	< 0·001	237·8 ± 17·7	< 0·001	< 0·001
C3	97·9 ± 12·5	116·1 ± 15·9	< 0·001	112·1 ± 17·8	< 0·001	120·0 ± 12·7	< 0·001	NS
C4	21·2 ± 4·1	30·0 ± 5·6	< 0·001	26·9 ± 4·9	< 0·001	33·2 ± 4·3	< 0·001	< 0·001

Lactoferrin (μg L^{-1}); TBA, thiobarbituric acid (μmol L^{-1}); ceruloplasmin (mg dL^{-1}); transferrin (mg dL^{-1}); TAS, total plasma antioxidant capacity (mmol L^{-1}); elastase (μg L^{-1}); α_1-antitrypsin (mg dL^{-1}); α_2-macroglobulin (mg dL^{-1}); fibrinogen (mg dL^{-1}); ESR, erythrocyte sedimentation rate (mm in the first hour); CRP, C-reactive protein (mg dL^{-1}); haptoglobin (mg dL^{-1}); C3 and C4 (mg dL^{-1}); C, control; IP, inactive psoriasis; AP, active psoriasis; NS, not significant.

(Fig. 5A); in 16 (53%) AP patients that ratio was higher than in IP patients (> 0·75). For elastase/α_1-antitrypsin (Fig. 5B), we found in 52 (87%) patients a value higher than in controls (> 0·60); 21 (70%) AP patients presented a value higher than in IP patients (> 0·87). Two groups were clearly defined for TBA/TAS ratio (Fig. 5C), as all patients presented a value higher than controls (> 1·97); moreover, we found that 18 (60%) AP patients presented a value higher than in IP patients (> 5·22).

As markers of neutrophil function/activation, we evaluated the values of elastase and lactoferrin per neutrophil (Table 3). We found significantly higher values in patients ($P < 0·001$, elastase/neutrophil; $P < 0·01$, lactoferrin/neutrophil). In IP and AP patients both ratios were significantly higher than in controls; the rise in AP was smaller than in IP for lactoferrin/neutrophil. Comparing IP vs. AP patients, a significantly higher value was found for elastase/neutrophil ($P < 0·001$), but not for lactoferrin/neutrophil. We observed (Fig. 6A) that all AP patients and 15 (50%) IP patients presented an elastase/neutrophil value higher than in controls (> 22·6); in addition, 20 (67%) AP patients presented a value higher than in IP patients (> 33·8). For lactoferrin/neutrophil no clear risk values could be established (Fig. 6B).

We also evaluated the correlations between all studied parameters (data not shown). We found for elastase more numerous and more highly significant correlations (Table 4). Elastase showed significant correlations with almost all the parameters studied.

Discussion

Psoriasis is a common and recurrent skin disorder, characterized by marked inflammatory changes in the epidermis and dermis. The histopathological study of active psoriasis lesions has revealed infiltration of WBCs, in particular of neutrophils. In the present study, we found a significantly higher WBC count (Table 1) in patients, resulting from an increased neutrophil number. In IP patients only a significant rise in neutrophils was observed. In AP patients both total WBC and neutrophils were significantly increased. Comparing IP vs. AP, we found that AP patients presented significantly higher values of WBC and of neutrophils.

An inflammatory response may last for minutes, hours or days, or it may turn into a chronic balanced process, or into a severe one. In the case of psoriasis, it may stay balanced or, unexpectedly, it may grow worse. We found that psoriasis was actually associated with inflammation (Table 2), as shown by the significantly higher levels of the inflammatory markers. In IP patients, these markers were significantly lower than in AP patients. Psoriasis presented as an inflammatory condition, and its worsening seemed to be linked to an enhanced or uncontrolled inflammatory response.

The inflammatory response, by generating chemotactic substances, triggers the mobilization and activation of the inflammatory cells,[1,8] namely the neutrophils, which may play a crucial role in the

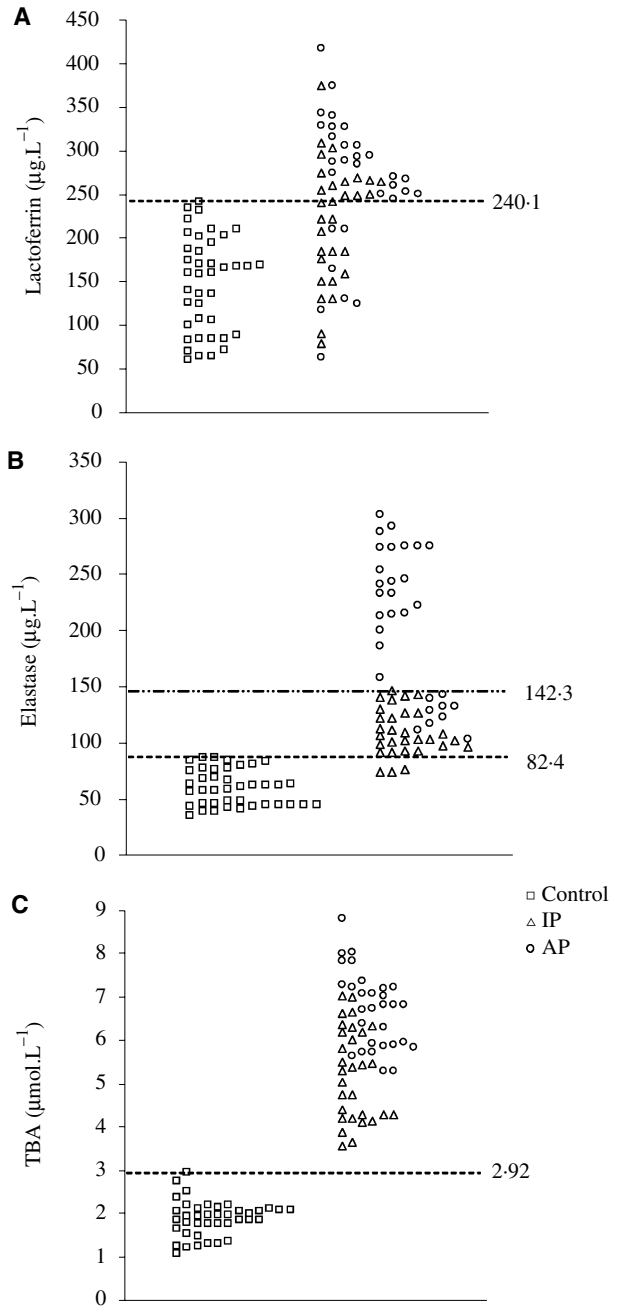

Figure 2. Plasma lactoferrin (A), elastase (B) and lipid peroxide (TBA, thiobarbituric acid) (C) levels in the 40 controls (□), in the 30 patients with inactive psoriasis (IP, △) and in the 30 patients with active psoriasis (AP, ○). The control values are under the line (- - -); the values in IP patients are under the line (- · · -).

clinical evolution of psoriasis. Their activation includes the release of the granule constituents[13] and a metabolic burst, producing ROS.[19] The increase in neutrophils in psoriasis seems to be linked to their activation, considering the observed rise in elastase and lactoferrin in psoriasis patients (Table 2). In IP, elastase was double the control value, and in AP it was almost four times that value. The rise in lactoferrin was less dramatic, being 1·5-fold higher in IP and twofold higher in AP. The plasma levels of elastase and lactoferrin should be influenced by the size of the neutrophil pool, and by its functional activity, shown by secretion of granule contents. The values of elastase and lactoferrin per neutrophil were therefore calculated for comparison of neutrophil number and neutrophil activation between patients and controls (Table 3). We found that the degranulation of the primary granules was stimulated in patients, as shown by a twofold increase in elastase/neutrophil. In AP, neutrophils seem to be hyperstimulated, as this ratio was almost 1·5-fold higher than in IP. A stimulated granulocytopoiesis is usually associated with immature circulating neutrophils displaying an increased volume of elastase-containing granules.[20,21] Hence, the observed rise in elastase/neutrophil may also reflect this change. The degranulation of the granules containing lactoferrin was also stimulated in patients, although to a lesser extent. Lactoferrin/neutrophil was higher in IP than in AP patients, although not significantly. High plasma levels of lactoferrin in psoriasis were reported by others.[12,22] However, none reported the values according to the severity of the disease and to neutrophil count. The biological function of high plasma levels of lactoferrin from neutrophils is still incompletely understood.[12] It is synthesized by granulocyte precursors and is stored in the specific granules, available for the response to infection and inflammation. It may act as a defence against ROS injury in its iron-free state, by complexing free iron, and as an enhancer of ROS production in its iron-loaded state, by providing catalytic iron.[12] Lactoferrin may also promote neutrophil adhesion and migration, representing a negative feedback modulator to prevent recruitment and activation of WBCs in inflammatory sites, by regulating cytokine release from mononuclear cells.[12,23] The lower value of lactoferrin/neutrophil in AP than in IP may result from a reduced degranulation or from a reduced lactoferrin content of specific granules. This could reflect the failure of the feedback mechanism of lactoferrin to control the enhanced inflammation.

Neutrophil granule subsets are mobilized under a specific order. Differences in degranulation content are due to quantitative differences (content or degranulation rate), and not to differences in the machinery controlling exocytosis of each granule

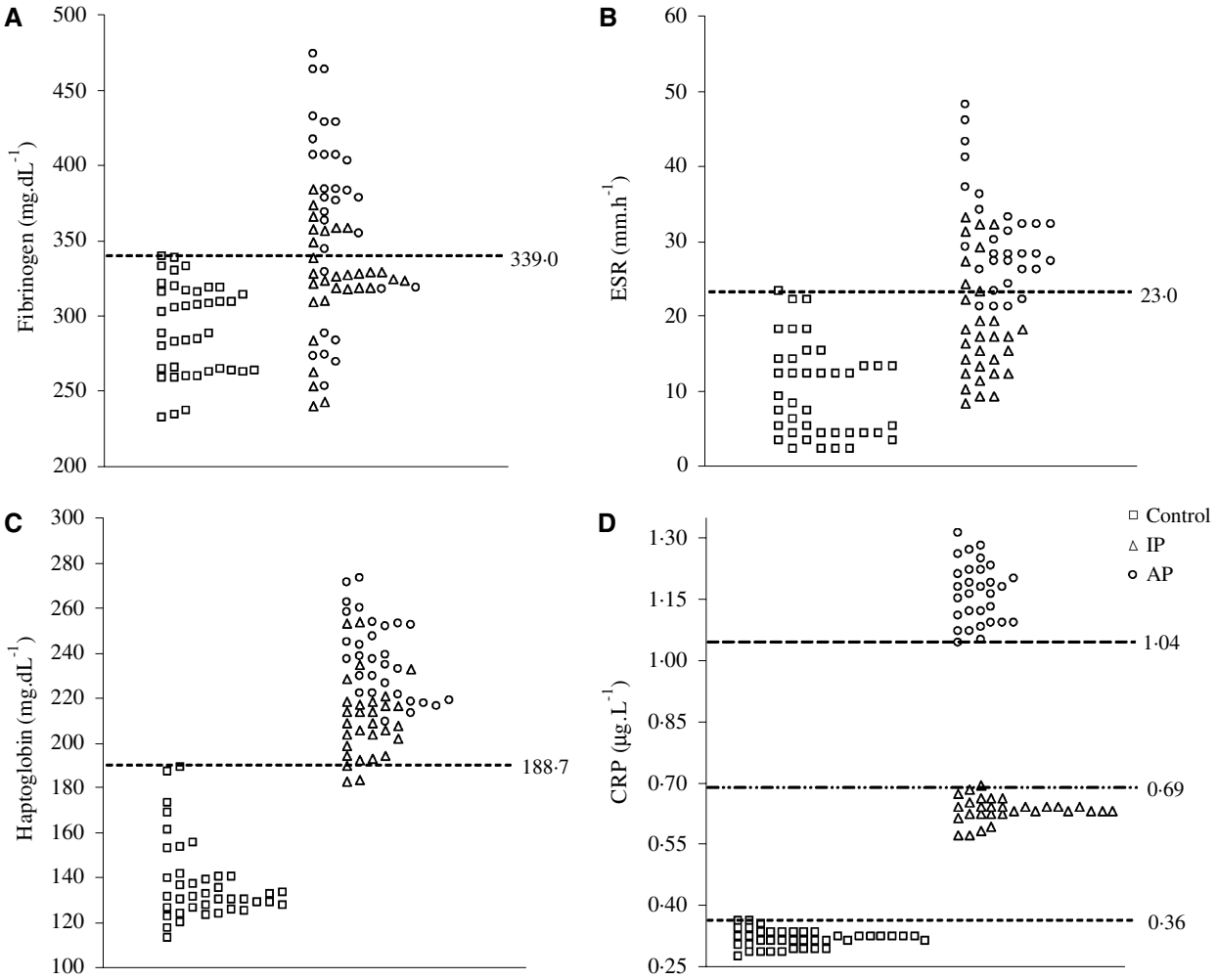

Figure 3. Levels of the inflammatory markers fibrinogen (A), erythrocyte sedimentation rate (ESR, B), haptoglobin (C) and C-reactive protein (CRP, D) in the 40 controls (□), in the 30 patients with inactive psoriasis (IP, △) and in the 30 patients with active psoriasis (AP, ○). The control values are under the line (- - -); the values in IP patients are under the line (- · · -); the values in AP patients are above the line (- - -).

subset.[8,13] The different changes observed in elastase/neutrophil and lactoferrin/neutrophil may result from a different mobilization rate of the granule subsets and/or from changes in their content. The reduction in lactoferrin content and/or in its secretion may represent the failure of the physiological mechanism to control the inflammatory response, and it also suggests the ability of neutrophils to interact with their environment.

The production of ROS was indirectly assessed by the lipid peroxidation levels (Table 2). We found a threefold higher value in patients, strengthening the previous results suggesting neutrophil activation. We also found that lipid peroxidation was significantly higher in AP patients than in IP patients.

Lactoferrin, ceruloplasmin and transferrin, all iron-linking proteins, were higher in AP than in IP; however, those levels may not avoid the development of the Fenton reaction, as suggested by the striking rise in lipid peroxidation, and the clear reduction in antioxidant defences observed in AP, when compared with IP.

The release of neutrophil activation products has to be counterbalanced by well-defined endogenous systems, to reduce or to avoid the enhancement of inflammation. We found in patients (Table 2) a significant reduction in TAS and an upregulation of α_1-antitrypsin and α_2-macroglobulin needed to reduce the deleterious effects of granular proteases such as elastase. The rise in lipid peroxides was not followed by

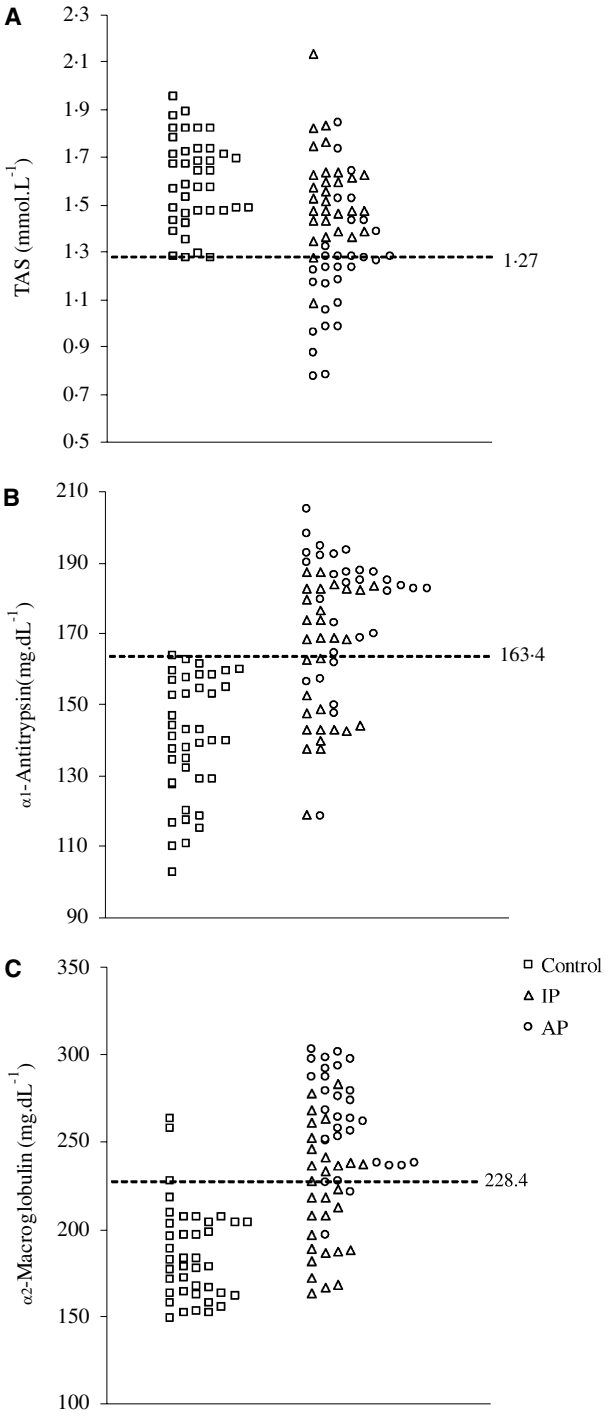

Figure 4. Levels of the inhibitors of leucocyte activation products total plasma antioxidant capacity (TAS, A), α_1-antitrypsin (B) and α_2-macroglobulin (C) in the 40 controls (□), in the 30 patients with inactive psoriasis (IP, △) and in the 30 patients with active psoriasis (AP, ○). The control values are above the line (- - -) for TAS (A) and under the same type of line for α_1-antitrypsin (B) and α_2-macroglobulin (C).

Table 3. Balance between products of neutrophil activation and their inhibitors, and the ratios of elastase and lactoferrin/neutrophil (mean ± SD) for control and psoriasis patients

	C ($n = 40$)	IP + AP ($n = 60$)	*P*-value (IP + AP)/C	IP ($n = 30$)	*P*-value IP/C	AP ($n = 30$)	*P*-value AP/C	*P*-value IP/AP
TBA/TAS	1·18 ± 0·31	4·60 ± 1·83	< 0·001	3·48 ± 0·93	< 0·001	5·72 ± 1·82	< 0·001	< 0·001
Elastase/α_1-antitrypsin	0·39 ± 0·12	0·89 ± 0·33	< 0·001	0·65 ± 0·10	< 0·001	1·14 ± 0·29	< 0·001	< 0·001
Elastase/α_2-macroglobulin	0·30 ± 0·09	0·63 ± 0·24	< 0·001	0·49 ± 0·11	< 0·001	0·78 ± 0·24	< 0·001	< 0·001
Elastase/neutrophil	14·2 ± 4·1	28·9 ± 8·6	< 0·001	23·0 ± 4·5	< 0·001	34·8 ± 7·7	< 0·001	< 0·001
Lactoferrin/neutrophil	37·9 ± 14·4	46·5 ± 14·0	< 0·01	48·2 ± 16·4	< 0·01	44·8 ± 11·2	< 0·05	NS

TBA, Thiobarbituric acid; TAS, total plasma antioxidant capacity; C, control; IP, inactive psoriasis; AP, active psoriasis; NS, not significant.

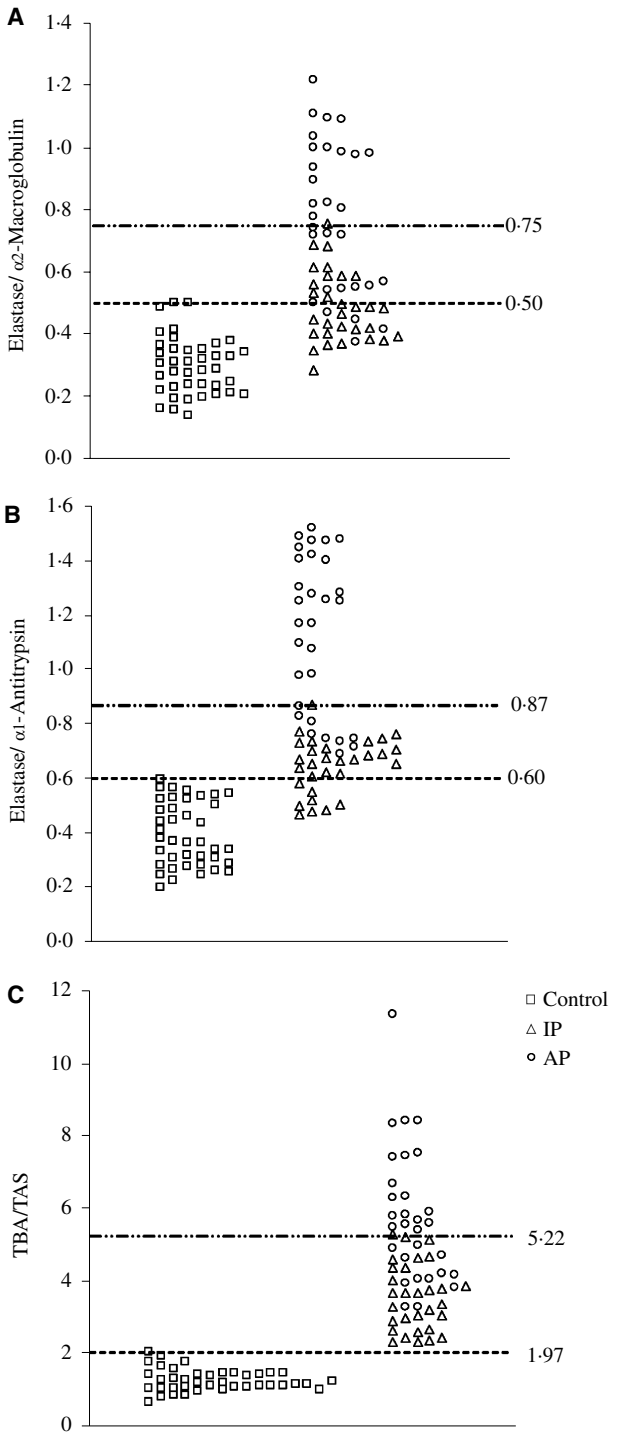

Figure 5. Balance between leucocyte activation products and their inhibitors elastase/α_2-macroglobulin (A), elastase/α_1-antitrypsin (B) and thiobarbituric acid/total plasma antioxidant capacity (TBA/TAS, C) in the 40 controls (□), in the 30 patients with inactive psoriasis (IP, △) and in the 30 patients with active psoriasis (AP, ○). The control ratios are under the line (- - -); the values in IP patients are under the line (- · · -).

a similar change in TAS, suggesting the development of oxidative stress. In IP, TAS was similar to control values, but a significant reduction was found in AP, suggesting its depletion.

For a more accurate study of the oxidative and proteolytic stress in patients, we evaluated the balance between elastase and their inhibitors, as well as between lipid peroxidation and total antioxidant defences (Table 3). We found significant imbalances (more than twofold the control value), showing the development of oxidative and proteolytic stress. In AP, these ratios were almost twofold higher than in IP.

An imbalance in the protease–antiprotease system, with uncontrolled proteolysis by elastase, was proposed to underlie degenerative and degradative disorders.[15] Elastase has been found in psoriatic lesions[5] and its activity was associated with scaling and inflammatory activity.[6,24] The imbalances we observed in patients, between elastase and its inhibitors, suggest that it may be seriously involved in spreading of the lesions. Strengthening this, the correlations between all the studied parameters (data not shown) showed for elastase more numerous and more highly significant correlations (Table 4). Hence, besides its crucial role in the worsening of psoriasis, it may provide a marker for monitoring the disease.

We may assume that in IP there is a continuous inflammatory process, underlying a sustained neutrophil activation and an oxidative and proteolytic stress. Suddenly, this apparently controlled form of psoriasis may turn into a severe form. We considered that it was important to analyse the results and to search for values of risk for worsening of psoriasis (Figs 1–6). We found that neutrophil count was more meaningful than WBC count (Fig. 1), as only in 30% of AP patients was the WBC count higher than in controls, whereas 63% of AP patients (and 13% of IP patients) showed a neutrophil count higher than in controls. Concerning neutrophil activation products (Fig. 2), we propose TBA as a marker for psoriasis, as all patients showed a value above the controls. Elastase also may provide a marker for psoriasis and for its worsening, as 95% of patients showed a value above the controls, and in 70% of AP patients the values were beyond the values in IP patients.

Among inflammatory markers (Fig. 3), haptoglobin and CRP appeared as markers for psoriasis, as 97% and 100% of patients, respectively, showed values higher than in controls. However, only CRP seems to provide a

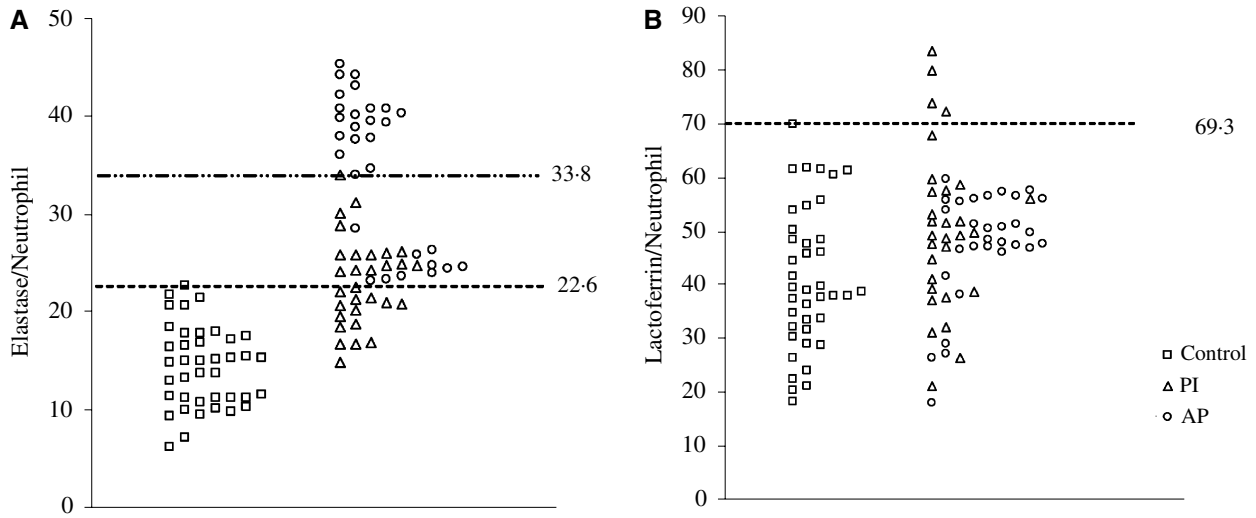

Figure 6. Neutrophil function as measured by the ratios elastase/neutrophil (A) and lactoferrin/neutrophil (B) in the 40 controls (□), in the 30 patients with inactive psoriasis (IP, △) and in the 30 patients with active psoriasis (AP, ○). The control ratios are under the line (- - -); the values in IP patients are under the line (- · · -).

Elastase	IP + AP	IP	AP
WBC	0·617; $P < 0·001$	NS	0·589; $P < 0·001$
Neutrophils	0·799; $P < 0·001$	0·457; $P < 0·001$	0·847; $P < 0·001$
Lactoferrin	0·616; $P < 0·001$	0·391; $P < 0·001$	0·752; $P < 0·001$
α_1-antitripsin	NS	0·653; $P < 0·001$	0·789; $P < 0·01$
α_2-macroglobulin	0·573; $P < 0·001$	0·219; $P < 0·001$	0·357; $P < 0·001$
TBA	0·418; $P < 0·001$	NS	NS
Ceruloplasmin	0·502; $P < 0·001$	0·535; $P < 0·001$	0·595; $P < 0·001$
Transferrin	NS	NS	NS
TAS	NS	NS	NS
Fibrinogen	0·760; $P < 0·001$	0·472; $P < 0·001$	0·803; $P < 0·001$
ESR	0·655; $P < 0·001$	0·290; $P < 0·001$	0·450; $P < 0·001$
CRP	0·795; $P < 0·001$	0·350; $P < 0·001$	0·623; $P < 0·001$
Haptoglobin	0·669; $P < 0·001$	0·241; $P < 0·001$	0·526; $P < 0·01$
C3	0·516; $P < 0·001$	0·560; $P < 0·05$	0·657; $P < 0·001$
C4	0·702; $P < 0·001$	0·471; $P < 0·001$	0·643; $P < 0·001$

Table 4. Correlation of elastase with the other studied parameters for psoriasis patients

WBC, White blood cells; TBA, thiobarbituric acid; TAS, total plasma antioxidant capacity; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IP, inactive psoriasis; AP, active psoriasis; NS, not significant.

marker for worsening of psoriasis, as all AP patients showed CRP values above those in IP patients.

Once elastase was identified as a marker for worsening of psoriasis, it was reasonable to expect α_1-antitrypsin and α_2-macroglobulin to be similar markers. However, they appeared only as markers for psoriasis, rather than its worsening (Fig. 4), suggesting an imbalance between elastase and its inhibitors. Indeed, their ratios (Fig. 5) were found to be higher than in controls in most cases (60% for elastase/α_2-macroglobulin; 87% for elastase/α_1-antitrypsin; 100% for TBA/TAS). These ratios seem also to provide markers for worsening of psoriasis, as most AP patients showed values higher than in IP patients (53% for elastase/α_2-macroglobulin; 70% for elastase/α_1-antitrypsin; 60% for TBA/TAS).

Neutrophil function, as given by elastase/neutrophil, seems to be a marker for psoriasis, as all AP and half of IP patients showed a value higher than in controls; it seems also to be a marker for worsening of psoriasis, as 67% of AP patients showed a value beyond the highest value in IP.

Further studies are needed to strengthen the prognostic significance of the proposed markers for

worsening of psoriasis (elastase > 142·3 μg L^{-1}; CRP > 0·69 mg dL^{-1}; elastase/α_2-macroglobulin > 0·75; elastase/α_1-antitrypsin > 0·87; TBA/TAS > 5·22; elastase/neutrophil > 33·8). We believe they could be useful in the prognosis of psoriasis, by traducing in advance its worsening. Clinicians would be able to initiate an adequate therapy earlier, avoiding or minimizing the worsening of psoriasis. Moreover, the markers could contribute to reducing the psychological impact of psoriasis, which is as debilitating as the spreading of the lesions.[25] They could also be used to monitor therapy, by giving information about its success. It would be of particular interest if they could give that information before visualization of remission of skin lesions, avoiding unnecessary overtreatment, or even unnecessary changes to a more aggressive therapy.

In summary, our data show psoriasis to be an inflammatory condition in which neutrophils seem to play a crucial role by contributing to the development of oxidative and proteolytic stress. The worsening of psoriasis seems to be linked to an imbalance between neutrophil activation products and their inhibitors. We propose that the insufficiency of the antioxidant defences and of the antiprotease system to face the enhanced release of neutrophil activation products may lead to an uncontrolled inflammatory process. Several conditions may trigger the enhancement of psoriasis, such as infections,[26] skin traumas and stress conditions.[4] All of them must trigger in IP an additional inflammatory stimulus, which may disrupt the fragile balance between the inflammatory products and the counterbalancing endogenous systems.

Epidermal hyperproliferation and inflammation are the main features in psoriasis. In our study we tried to characterize further the inflammatory response in IP and AP, in order to find potential prognostic markers of worsening or improvement of psoriasis, by establishing the differences between both forms of psoriasis. We wonder if the observed changes are a cause or an effect of the disease, or both. In IP the changes are probably an effect of the disease; however, when an additional inflammatory response is needed, it may be the cause for worsening of psoriasis by the imbalance caused, but in addition, ultimately, also an effect of the disease.

A great deal of research is underway on psoriasis and on new therapies.[15,27–31] Our data, by stressing the role of neutrophil activation products and of their inhibitors, the antioxidant defences and the antiprotease system, support therapeutic research in these areas.

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