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Laboratório de Genética

Psoriasis is more than a skin disorder. An ACE study

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Abstract

Introduction: Psoriasis is now known to be a systemic autoimmune disease with an associated increased cardiovascular risk. This seems to rely on systemic inflammation. Angiotensin-converting enzyme (ACE) has been associated with oxidative stress, a state characteristic of cardiovascular disorders and this enzyme's activity is higher in psoriasis. Our aim is to assess cardiovascular risk in psoriatic patients and its association with ACE polymorphism and activity.

Methods: Our population consisted of 1155 individuals (64 psoriatic patients, 1091 controls) whose ACE genotype and activity were determined. The cardiovascular risk was estimated through lipid profiles. PASI score was used as a severity measurement.

Results: Psoriatic severity was associated with a higher cardiovascular risk estimated by lower HDL concentrations ($r=-0.496$, $p=0.007$) and higher triglyceride levels ($r=0.421$, $p=0.020$), as well as TC/HDL and LDL/HDL ratios ($r=0.612$, $p<0.001$ and $r=0.437$, $p=0.023$, respectively) which have been shown to have stronger predictive value for cardiovascular disorders

Conclusions: Psoriatic patients should be treated as a risk group for cardiovascular diseases and new markers for severity and this associated risk may be of worthy pursuit. Further studies are crucial to enhance our knowledge on inflammation and psoriasis.

Keywords: ACE, Psoriasis, Cardiovascular Risk, Lipid profile

Resumo

Introdução: A psoríase é atualmente vista como uma doença sistémica autoimune com um risco cardiovascular associado, cuja correlação parece advir de um estado de inflamação sistémica. A enzima conversora de angiotensina (ECA) associa-se a *stress* oxidativo, característico da patologia cardiovascular e apresenta níveis de atividade superiores na psoríase. O nosso objetivo é avaliar este risco e a sua associação com o polimorfismo e atividade do ECA.

Métodos: A amostra consiste em 1155 indivíduos (65 psoriáticos, 1091 saudáveis), dos quais se determinou o genótipo do ECA e a sua atividade. O risco cardiovascular foi estimado a partir do perfil lipídico e a severidade da doença pelo índice PASI.

Resultados: A severidade da psoríase associou-se a diminuição do HDL ($r=-0.496$, $p=0.007$) e aumento dos triglicéridos ($r=0.421$, $p=0.020$), bem como dos índices CT/HDL e LDL/HDL ($r=0.612$, $p<0.001$ e $r=0.437$, $p=0.023$, respetivamente), os quais traduzem melhor valor preditivo para doenças cardiovasculares.

Conclusões: Os doentes psoriáticos devem ser tratados como um grupo de risco para patologia cardiovascular e deverão ser procurados novos marcadores para a severidade da doença e risco associado. Novos estudos são essenciais para melhorar o conhecimento nesta área.

Palavras chave: ECA, Psoríase, Risco Cardiovascular, Lípidos

O Trabalho Final exprime a opinião do autor e não da FML.

Psoriasis is more than a skin disorder. An ACE study.

Introduction

Psoriasis affects approximately 2% of the population worldwide [1, 2], accounting for 12 million individuals within the USA and Europe [3], with a higher prevalence at higher latitudes and also more prevalent among caucasians [4].

Psoriasis can present at any age and in different ways. It can be classified according to morphologic appearance including plaque, inverse, erythrodermic, pustular, and guttate forms, sometimes with nail manifestations as well, including nail dystrophy and psoriatic lesions of the nail bed [2, 5]. The most common clinical variant is psoriasis vulgaris, representing 90% of all cases. This type of psoriasis is usually manifested as well circumscribed red or salmon pink papulosquamous plaques covered by silvery scales surrounded with normal skin. Manifestations are generally symmetrically distributed in the body, specially on the extensor area of the elbows and knees, on the scalp, and lumbosacral region [1, 6], although any or all portions of the body surface may be affected.

Patients with psoriasis have a higher prevalence of numerous comorbidities, such as pulmonary, renal, hepatic, rheumatologic and cardiovascular diseases [7] with a clear majority of the literature revealing a correlation between psoriasis and cardiovascular risk, linking it with disorders such as obesity, hypertension, dyslipidemia, diabetes mellitus (DM) and metabolic syndrome. This epidemiologic association seems to be stronger as psoriasis is more severe [8]. Even though there are multiple definitions of disease severity, it is accepted by most that mild psoriasis has less than 3% body surface area involved, moderate psoriasis between 3 and 10% and severe psoriasis more than 10% body surface area involvement, as proposed by the National Psoriasis Foundation [9].

Obesity, a well known cardiovascular risk factor, has been studied as a comorbidity in patients with psoriasis and the association has been shown to grow with the severity of psoriasis, with an adjusted odds ratio (OR) of 1.79 (95% CI [1.55,2.05]) for patients with severe psoriasis and 1.27 (95% CI [1.24,1.31]) among the ones with mild psoriasis [10]. Despite there being a definite connection between the two it is not yet established whether obesity predates or comes after the development of psoriasis. Just as the definition of OR itself explains, it does not establish a cause-effect relation in either direction and it could even be that the association is due to a third property,

which is a contributing factor for both psoriasis and obesity, or even a common underlying pathophysiology. In fact there is both evidence of a BMI increase in patients after they were diagnosed with psoriasis [11], pointing to obesity as secondary to the disease, and strong evidence that indicate that obesity is a risk factor for the development of psoriasis, with both hip circumference and waist-hip ratio associated with higher risk of psoriasis [12, 13].

This “psoriasis’ obesity” is believed to be an important connection to all other cardiovascular disorders and risk factors among these patients. There is even a tendency for central adiposity as shown in a study which illustrated that children and adolescents with psoriasis are more prone to obesity and that this obesity is more likely abdominal [14], and waist circumference, waist-hip ratio or any other central adiposity measures are presumed to be more relevant to cardiovascular risk than an increased BMI [15, 16]. In a 24 week study there was much more significant results in reducing disease severity when cyclosporine therapy was combined with a low-calorie diet as opposed to cyclosporine alone [17], suggesting that obesity may also affect treatment efficacy.

Pathophysiologically, psoriasis seems to share many cytokines with obesity, such as TNF- α and IL-6. There is a shown rise of both serum TNF- α levels and TNF- α receptors as BMI and waist circumference increase [18] and there is also higher levels of c-reactive protein (CRP) in psoriatic populations as well as a positive association between its levels and BMI [19], being not only increased in active stages of disease but also during remission compared to controls [20].

In regards to hypertension, there have been studies that suggest a correlation between psoriasis and hypertension as well as the difficulty to control it [21]. This correlation seems to be severity dependent [22].

Dyslipidemia has also been associated with psoriasis. Triglyceride, total-cholesterol, and LDL-cholesterol levels were found to be higher in psoriatic patients [23, 24], and these changes were seen at the onset of the disease [25], leading to the possibility that dyslipidemia may precede psoriasis. It is however unclear whether this lipid profile changes are due to the increased incidence of obesity in psoriatic populations and its influence in lipid metabolism or actually independently associated with psoriasis. Adipocytes release a variety of pro inflammatory molecules including some cytokines such as TNF- α and IL-6. These molecules are shown to not only increase cardiovascular risk [26] but also to affect free fatty acids, cholesterol and lipid levels [27]. There have been a few studies that found an atherogenic profile with decreased total HDL levels and higher levels of small LDL and HDL particles in psoriatic patients, measured by nuclear magnetic resonance spectroscopy, and these were shown to be associated with increased aortic inflammation,

which suggests that HDL particle characteristics may be an important participant in the cardiovascular risk seen in psoriasis [28, 29]. The same study found a lower HDL efflux capacity in psoriatic patients, which is associated with non calcified coronary atherosclerosis, non related to the HDL levels [30]. This decrease in cholesterol efflux may be explained by another study that describes psoriatic HDL as having diminished phospholipid and cholesterol components, which results in the reduction of its capacity to promote cholesterol efflux from macrophages. This efflux capability was negatively correlated with psoriasis area and disease severity [31].

Another important comorbidity is the metabolic syndrome, that in a way correlates most of the comorbidities discussed above. It is directly linked to intra-abdominal obesity, and is perceived as a setting of chronic systemic inflammation that meets at least three of the following: abdominal obesity, impaired glucose regulation, hypertriglyceridemia, reduced HDL and hypertension [32]. The association has been proved in many studies, with abdominal obesity as the most common criteria met by the psoriatic group, followed by hypertriglyceridemia and decreased levels of HDL [33].

This abdominal obesity must be recognized almost as an endocrine organ that is in fact able to release many bioactive peptides (adipokines) such as TNF- α , IL-6, leptin and adiponectin that coordinate inflammation, glucose and lipid metabolism and endothelium function [34].

Adipocytes may also interact directly with macrophages that are increased in the dermis and which, by releasing TNF- α , will influence gene transcription in adipose tissue, augmenting the levels of leptin in decrement of adiponectin levels which is an important anti-inflammatory molecule that also functions in regulating insulin sensitivity, and block insulin signaling, becoming a major contribute for insulin resistance [35]. This insulin resistance was shown to be present in the dermal endothelium of both psoriatic plaques and “normal” skin in patients with psoriasis and it promotes endothelial expression of adhesion molecules and inflammation by shifting anti-/pro-atherogenic insulin-dependent pathways towards the latter [36]. Furthermore, it has also a role in regulating keratinocytes, since some of the cytokines that cause this shift and the consequent insulin resistance, namely IL-1 β , are also responsible for disrupting insulin-dependent keratinocyte differentiation and thus boosting keratinocyte proliferation [37], one of the most discussed features of psoriatic skin.

TNF- α , besides inducing insulin resistance, increasing production of adhesion molecules by endothelial cells and having a positive correlation with BMI as discussed above, also augments free fatty acids which further induce insulin resistance, all of which are crucial steps for endothelial damage and atherogenesis [38]. Additionally, IL-6 too induces insulin resistance and increases

adhesion molecules production. It also stimulates the liver to release fibrinogen and CRP, all of which further encourage atherosclerosis [39].

Adiponectin seems to work against these actions, augmenting insulin sensitivity, decreasing TNF- α production and monocyte cell adhesion and reducing transformation of macrophages into foam cells [40], which is a feature of atherosclerosis [41]. It is positively correlated to weight loss and high levels of adiponectin have been shown to confer lower risk for myocardial infarction (MI) in men [42]. This adipokine's plasma levels seem to be negatively correlated with BMI, as opposed to leptin, which increases with both BMI and waist circumference. Adiponectin decreases more in relation to visceral adiposity than to subcutaneous adipose tissue [43]. Adiponectin was also found to be decreased in hypertension and ischemic heart disease [44, 45].

Leptin, on the other hand, seems to have opposite effects. In a study that compared different ethnicities, individuals at high risk for cardiovascular diseases and metabolic syndrome had higher levels of plasma leptin [46], which goes hand to hand with a study that found increased leptin to affect carotid intima-media thickness (CIMT) [47]. Leptin was also shown to be higher in patients with psoriasis, working as a severity marker [48].

Finally, another comorbidity that has been associated with psoriasis is type II diabetes. A recent study confirmed that patients with psoriasis had higher rates of DM and this higher risk was once again severity dependent [49], as it was shown before [10, 50]. This association could be due to the many insulin resistance mechanisms explained before, mainly driven by TNF- α , however psoriasis seems to be associated with a diabetes susceptibility locus which might suggest a stronger correlation from the base [51], and the association between psoriasis and DM is shown to still be present when adjusted for BMI [52].

Psoriasis has been, as discussed above, linked to various comorbidities and it's also associated with an increased mortality, specially in cases of severe psoriasis [53, 54]. This increased risk of death has been said to have numerous etiologies including infection, kidney disease and dementia however to be mainly due to cardiovascular problems [55] and markedly seen in young patients with severe psoriasis in which the risk of MI is significantly higher. [56]

It is also important to note the association between psoriasis and both CIMT and coronary artery calcification (CAC), which seem to be good parameters when in search for a cardiovascular risk measurement [57-60]. A study in 2016 showed a significant adjusted association between CIMT and PASI (psoriasis area and severity index), which is a score of affected body area and

severity in psoriasis [61]. The mean CAC was also shown to be increased in other studies when comparing psoriatic patients to controls [62].

One of the mechanisms proposed to link psoriasis and cardiovascular disorders is inflammation and despite the fact that, for a long time, psoriasis was seen as a skin disease, there has been growing and now significant evidence of it being a systemic disorder with an underlying systemic inflammatory process. We should therefore perceive psoriasis as a common, chronic systemic disease most frequently manifested by skin lesions [5].

Inflammation has been associated with cardiovascular risk, on one hand because it plays a central role in atherosclerosis, which is another mechanism that has been conjectured as a link between psoriasis and cardiovascular diseases [50], and on the other hand because it has been suggested that chronic inflammation itself may lead to an exacerbation of cardiovascular complications, with a possibility that this effect might actually work through vascular damage as well. A study using murine models with psoriasiform dermatitis without any comorbidities showed aortic root inflammation in 33% of the affected group compared with 0% in controls ($p=0.04$) and concluded that skin-specific inflammation promotes both vascular inflammation and an increase in thrombotic rates and that treatment against this skin inflammation attenuates these pathways [63]. In fact, systemic treatments targeting psoriatic underlying inflammation have been studied and shown to improve both psoriasis and the cardiovascular disorders. Methotrexate was proven to significantly reduce vascular disease risk, specially when given in lower doses [64], which could be explained by the fact that in lower doses methotrexate's anti-inflammatory properties might outweigh its influence in homocysteine levels, that are shown to augment cardiovascular risk [65, 66], supported by the same study in which the association of folic acid to the high dose treatment improved the outcomes as well [64]. There was also a study in which etanercept had a lowering influence on CRP levels [19] and this inflammatory protein has been shown to add prognostic information in the prediction of future cardiovascular events beyond that given by all lipid measures [67]. In 2011, another study demonstrated that an anti-psoriatic treatment improved endothelial cell dysfunction [68].

Atherosclerosis has an undeniable bond with psoriasis as it is significantly more prevalent in psoriatic patients with an adjusted OR (for gender, race, BMI, smoking status, hypertension, HDL and CRP) of 2.67 (95% CI [1.20,5.92]), having an even stronger association when adjusted for age, since there is an excess of atherosclerosis in younger psoriatic groups [69], and because both plaques have similar inflammatory cell infiltrates in their content [70]. Psoriasis is a Th1, Th17 and Th22 inflammatory disease, with expansion and activation of these T cells subsets, antigen

presenting cells and the subsequent cytokines [71, 72] and these Th1 and Th17 cascades are common to atherosclerosis [70]. The activation of these pathways and its cytokines such as IL 17 and IL 6, increased in psoriasis, also help further progression of atherosclerotic plaques and are accordingly found increased in cases of unstable coronary artery disease [73, 74]. Monocytes, that are known to promote arterial smooth muscle proliferation and transformation of macrophages into foam cells within the vessel wall [75, 76] have an increased aggregation that is characteristic of psoriatic inflammation [77]. Furthermore, raised levels of monocyte chemoattractant protein-1 and macrophage-derived chemokine are associated with cardiovascular risk, specifically atherosclerotic plaque instability and rupture [78] and are found increased in skin lesions and serum of psoriatic patients [79].

Insulin resistance is yet another mechanism suggested to contribute to cardiovascular diseases in psoriasis, however it is thought to be induced by chronic inflammation [80] and it seems to play a massive role in atherosclerosis [81, 82], so it might just be a part of the same extensive mechanism where changes like insulin resistance, atherosclerosis and dysregulation of adipose tissue, that lead to the many comorbidities discussed above, are driven by cytokines and other pro-inflammatory mechanisms, which appear to be over regulated in psoriasis. These changes seem to further amplify inflammation, creating a sort of vicious cycle between psoriasis and cardiovascular diseases [83].

A relevant and currently discussed part of psoriasis and its development is the genetic background. Besides the commonly discussed genes involved in psoriasis (PSORS1-13, D2S134) [84] there seem to be different genetic factors that contribute to disease susceptibility [85]. One example of these might be the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism.

ACE is a carboxypeptidase that cleaves angiotensin I into the vasoactive angiotensin II in the endothelium surface and that also degrades bradykinin, a strong vasodilator, providing a double vasoconstrictive effect. Angiotensin II increases formation of reactive oxygen species, stimulates the synthesis of cytokines including IL6 and IL8 [86, 87], which are present in the pathogenesis of both psoriasis and cardiovascular diseases as discussed previously, and promotes insulin resistance [88], which suggests that higher levels of ACE would be in concordance with the pathophysiology of psoriasis. On the other hand, bradykinin has a pro-inflammatory effect as well, enhancing synthesis of IL6 and IL8 [89], indicating that lower levels of ACE or even ACE inhibition could favor the inflammatory state seen in psoriasis. ACE-inhibitors have in fact been associated with

exacerbations and even appearance of psoriasis [90, 91]. Favoring the first, higher ACE levels suggest a state of oxidative stress, characteristic of cardiovascular disorders [92].

The ACE gene is located on chromosome 17q23 and has 26 exons and 25 introns. On intron 16 a polymorphism was detected, defined by an insertion or a deletion of a 287 bp non coding Alu repeat sequence [93] that has been shown to affect ACE levels and function [94]. Serum ACE concentrations were found higher in DD homozygotes, with the I/D polymorphism accounting for 47% of the variability in these concentrations [95].

Multiple studies showed an increment in serum ACE activity in patients with psoriasis when compared to healthy controls [96], which decreased to identical levels as those seen in controls after treatment for psoriasis. Various psoriatic treatments were studied and there was no significant difference on how ACE levels dropped in each case [97], suggesting that ACE could be an useful measurement for therapeutic efficacy.

A study using murine models showed that inflammation reduced the ACE2/ACE ratio, with a clear decrease in ACE-2 levels and a trend to increase ACE gene expression [98], pointing to the possibility that higher levels of ACE in patients with psoriasis could be due to the inflammatory state that this disease involves [99] and the consequent shift in the ACE2/ACE balance, which is also favored by the fact that any type of treatment decreased ACE levels to levels identical to those seen in healthy controls. ACE-2 was discovered to balance the pro-inflammatory and vasoconstrictive effects of the ACE/angiotensin II/AT1 axis, through the ACE2/angiotensin-(1-7)/Mas counter-regulatory axis [100].

Because of the seen alterations in ACE levels in psoriatic patients and also the common pathways that seem to exist between the two, its polymorphism has been studied in this population. Studies are conflicting in the matter, showing either no association between the ACE I/D polymorphism and the risk of psoriasis [101], or contradictory results regarding which genotype is associated with a risk for psoriasis:

- within the Asian ethnicity a few studies show that the ACE II genotype and I allele might confer susceptibility to psoriasis [102, 103];
- studying a Turkish population, the distribution of the ACE I/D polymorphism didn't have significant differences in psoriatic patients when compared to controls, nonetheless the I allele was found to be significantly associated with familial psoriasis. Furthermore, onset was earlier in patients with familial psoriasis carrying the I allele [104];

- in an Austrian study, the analysis of all psoriatic patients showed a significantly higher prevalence of the II genotype than in controls, with a stronger association when restricting the study to patients with early-onset psoriasis [105];
- a greek study found, however, the D allele and ID genotype to be more common in patients with early onset psoriasis [106].

In terms of cardiovascular risk, besides the well-known factors such as smoking, hypertension and DM, there are individual inherited factors that play a role in the development of cardiovascular diseases, which is supported by the fact that only a fraction of patients with said risk factors have in fact a cardiovascular event. The ACE I/D polymorphism has been studied and proposed to be one of these inherited factors. One study with a 518 patient population showed a clear association between the D allele and a higher rate of major cardiovascular events with a hazard ratio of 1.64 (95% CI [1.27,1.98] $P < 0.001$) [107]. The DD genotype was also found to be associated with an increased risk of left ventricular hypertrophy [108] and both the DD and the ID genotypes have shown significant associations with the development of coronary artery disease [109].

This study's purpose is to correlate the cardiovascular risk in psoriasis with the ACE I/D polymorphism and more importantly this enzyme's activity levels, whose association to psoriasis despite having conflicting results may be one of the genetic and molecular factors that significantly influences cardiovascular risk within this group of patients. In order to assess the cardiovascular risk, as many have done before, the lipid profile was used with its many parameters that have been shown to have a good predictive value regarding cardiovascular disorders [110]. In fact, abnormalities in lipoprotein metabolism are one of the key factors in atherogenesis (the basis for a great number of cardiovascular disorders), shown to represent around 50% of the modifiable risk of developing cardiovascular disease [111].

Methods

This study utilizes data from 64 patients with psoriasis, whose blood samples were analyzed by the genetics lab team from Faculdade de Medicina da Universidade de Lisboa and were granted for the purpose of this study by Ângela Gil, Masters in Human Biology and Genetics. These blood samples belong to patients with psoriasis from the Dermatology department in Hospital de Santa Maria - Centro Hospitalar Lisboa Norte, and were provided by Paulo Filipe, PhD MD and Joana Antunes, MD.

The control group includes data from 1091 individuals obtained in Clínica de Endocrinologia, Diabetes e Metabolismo de Lisboa and granted by Mário Rui Mascarenhas, PhD MD and Joana Ferreira, PhD student MD.

With the blood samples, DNA extraction, quantification and amplification were performed using a modified salting out method, the NanoDrop spectrophotometer, PCR with specific primers for the ACE polymorphism and the GeneAmp PCR System 2700 thermal cycler. Afterwards, a gel electrophoresis allowed a determination of the patients' genotype based on how the DNA fragments moved through the matrix of agarose reflecting their size (Restriction Fragment Length Polymorphism technique) and therefore whether that sample had two larger phenotype fragments as in the II genotype, two smaller ones (DD genotype) or one of each in the ID genotype (see attachment 1).

The enzyme activity was also determined by recording the decrease in absorbance of furylacryloyl-phenylalanyl-glycyl-glycine (FAPGG) at 346 nm for 15 minutes at 37°C, since ACE also mediates this substrate's cleavage.

In addition to the ACE genotype, the data collected from both the psoriatic and the control group included age, gender, BMI (only available for the control group), total cholesterol (TC), HDL, LDL and triglycerides with missing information in a few individuals. Further calculations were performed to obtain the nonHDL cholesterol levels (TC - HDL) and the LDL/HDL ratio. 50 of the 64 psoriatic patients also had their PASI score calculated.

The statistic analysis was performed with the IBM SPSS Statistics 24.

Results and discussion

This study's populations display a clear difference between the gender frequencies, with 76.7% males to 23.3% females in the control group and 29.7% males to 70.3% females in the psoriatic group. In order to surpass this discrepancy all tests comparing the two populations were either performed separately for female and male subjects or adjusted for gender. In terms of age, it is shown in table 1 that both groups are similar with a calculated non significant correlation using a t-test and therefore no major adjustment is required.

		Psoriatic patients	Controls
Age	<i>female</i>	53.18±13.03 (n=17)	54.71±13.95 (n=837)
	<i>male</i>	51.73±13.06 (n=40)	55.61±12.75 (n=254)
	<i>total</i>	52.16±12.95 (n=57)	54.92±13.68 (n=1091)

Table 1: analysis of the characteristic age with mean±standard deviation and number of cases

Table 2 shows the ACE genotype frequencies in the psoriatic and control groups. The control group is in the Hardy-Weinberg equilibrium as seen by the chi-square test ($\chi^2=0.718$, $p=0.698$). It was also checked for the psoriatic group which was likewise found to be distributed according to the equilibrium ($\chi^2=0.204$, $p=0.903$).

		Psoriatic patients	Controls
ACE I/D polymorphism	<i>II</i>	11.5% (n=7)	13.3% (n=59)
	<i>ID</i>	41.0% (n=25)	43.8% (n=194)
	<i>DD</i>	47.5% (n=29)	42.9% (n=190)
		$p=0.777$	
ACE allele	<i>I</i>	32.0% (n=39)	35.2% (n=312)
	<i>D</i>	68.0% (n=83)	64.8% (n=574)
		$p=0.480$	

Table 2: frequencies of the ACE polymorphism and alleles and respective number of cases; p-values from chi-square test; significance level defined as $p \leq 0.05$

In terms of genotype frequencies, the DD genotype appears to be more frequent in psoriatic patients. However, once a chi-square test is executed, no correlation is shown to be statistically significant. The same for the analysis of both alleles (also in table 2), with the D allele in higher frequency in the psoriatic group but, once again, the chi-square test not showing a significant correlation between them and the disease. This result, despite being negative, supports some of the data discussed above where a lack of consistency in results is predominant [101-106]. There is also no significant difference between both genders.

Once analyzing ACE activity instead of the genotype, this enzyme's activity is found to be significantly higher in female psoriatic patients, with a strong tendency to also be increased in male psoriatic patients when compared to controls (table 3). When adjusting for gender, the entire psoriatic population was still found to have significantly higher ACE activity levels than controls.

		Psoriatic patients	Controls	
ACE activity	<i>female</i>	17.38±7.67 (n=6)	11.62±6.59 (n=220)	p=0.037
	<i>male</i>	19.83±14.66 (n=14)	12.76±6.96 (n=56)	p=0.063
	<i>total</i>	19.09±12.80 (n=20)	11.85±6.67 (n=276)	p=0.015 p=0.049*

Table 3: means for ACE activity displaying the standard deviation and number of cases for each subgroup; p-values calculated with t-test or Mann Whitney test according to the distribution of the samples; significance level defined at $p \leq 0.05$; *p-value adjusted for gender with linear regression

When analyzed if the ACE activity was influenced by its genotype (table 4) using both psoriatic patients and controls, a higher activity level corresponded to the DD genotype and an intermediate level was found in the heterozygote population. As mentioned before, this result is in agreement with previous studies [94, 95], despite being also known that the genotype only accounts for about half of the variability of the enzyme's levels.

		ACE activity
ACE I/D polymorphism	<i>II</i>	7.75±3.31 (n=24)
	<i>ID</i>	11.27±5.62 (n=101)
	<i>DD</i>	14.57±8.70 (n=93)

p<0.0001

Table 4: ACE activity for each ACE genotype with mean±standard deviation and number of cases; p-value calculated with a kruskal-wallis test; significance level defined at $p \leq 0.05$

Once analyzing this correlation in both groups separately, the association appears to be stronger in the control group, which might support the idea that the increase in ACE activity in psoriatic patients compared to healthy subjects could be due to other mechanisms and not dependent on the ACE genotype which in fact seems to have similar frequencies in both groups. When using a two-way ANOVA test to check the influence of psoriasis adjusted for the genotype it is still significant ($p=0.004$). In conclusion, genetics seem to play a smaller role in the variability of ACE activity in individuals with psoriasis versus healthy subjects and this might be due to some kind of stimuli that maintains ACE levels higher in psoriatic patients regardless of their genotype.

A study in 2008 demonstrated using murine models that the skin, besides playing a known important role in the adaptation to environmental changes (with its vascular plasticity), is in fact a critical mediator of systemic responses to environmental oxygen [112]. This study showed a response to hypoxia through the epidermal transcription factor HIF-1 α which induces nitric oxide release to increase erythropoietin expression. Transposing this to psoriatic patients, it is possible that their skin suffers from hypoxia due to the cellular hyperproliferation (characteristic in this disease) which would then lead to the mentioned release of NO and could explain the differences seen in ACE activity when comparing to the control group. Higher ACE levels suggest an oxidative stress state which is present in many cardiovascular disorders and is in fact one of the precipitating factors for atherogenesis [92, 113].

Considering the lipid profile as the cardiovascular risk measurement for this study and pursuing the analysis of the possible association between ACE and cardiovascular disorders, only a weak correlation could be found between ACE activity and LDL/HLD ratio ($r=0.133$, $p=0.05$). No other correlation between this enzyme's activity and different cholesterol measures was found to be significant when analyzing all subjects.

The means of ACE activity are different when dividing the subjects into two groups considering the lipid concentration found to be the limit for pathologic levels. For example, looking at the subjects with a considered normal total cholesterol, a lower mean of ACE activity is found when comparing them to the subjects with a TC ≥ 190 mg/dl, and the same goes for the other lipid parameters. However, these differences in means are nonsignificant. It is felt that a larger psoriatic sample with their ACE activities measured would be of importance to assess the true influence of this enzyme in the cardiovascular risk seen in these patients.

In this study, more parameters within the lipid profile were measured, such as the mentioned LDL/HDL ratio, TC/HDL ratio and non-HDL cholesterol levels. It has been established that LDL cholesterol levels are not informative enough regarding the lipoprotein metabolism and these “atherogenic indices” have been defined in order to optimize this predictive capacity. LDL becomes even less reliable, in terms of its calculation, when triglyceride concentration exceeds 300mg/dl [110], which is true for a part of the individuals suffering from dyslipidemia. LDL may also underestimate the magnitude of the lipoprotein abnormality in these patients since it does not account for VLDL or IDL, which is where the non-HDL outweighs LDL predictive value [110].

Total cholesterol on its own is also a flawed indicator since it displays no difference between an increase in HDL cholesterol or an increase in LDL. Total cholesterol, HDL, and TC/HDL ratio were compared between healthy subjects and myocardial infarction survivors and the ratio was found to have less overlap of populations [114].

When it comes to HDL, reduced concentration levels mean reduced anti-atherosclerotic trait and are associated with various risk factors. It probably implicates independent risk [115] and is critical to assess, giving the ratios a good predictive value [110].

In conclusion, the weak correlation between ACE activity and LDL/HDL ratio may be more meaningful than is apparent, however the lack of other correlation with HDL, non-HDL and TC/HDL ratio makes it less likely that a clear association exists between ACE activity and a worse lipid profile.

The fact that the control group consists of a population of patients followed in an endocrinology clinic raises the question as to whether it is a good sample of the non-psoriatic population, since in terms of obesity, DM and hypertension this population may have a higher prevalence than the general population. The statistical analysis should therefore be adjusted for such pathologies.

Despite the lack of information regarding BMI in the psoriatic group, it is clear that the control group has a very large proportion of individuals with increased BMI. Once excluded the population with a $BMI \geq 30 \text{ kg/m}^2$, there is a tendency for an increase in nonHDL cholesterol with a higher ACE activity ($r=0.146$, $p=0.082$). In the same circumstances, the correlation between ACE activity and LDL/HDL ratio becomes slightly stronger ($r=0.209$, $p=0.016$). One would expect such modification considering the fact that obesity is an inflammatory disease as well as psoriasis and could therefore have a stronger influence on the cardiovascular risk masking the effect of psoriasis or in this case ACE levels. Even these levels may be influenced by the coexistence of obesity. In fact, in 2002, a study showed a decrease in serum ACE related to weight loss in obese subjects

[116]. Although these results are not significant since this variable was only accounted for in the control group, they are mentioned as an indication of the possible positive changes in this study's results if this exclusion criterion could be met for all subjects involved.

When trying to assess the differences in cardiovascular risk between psoriatic patients and controls, even though lower levels of HDL, higher TC/HDL and LDL/HDL ratios appear to be associated with psoriasis, once adjusted for gender and age these associations become statistically nonsignificant (table 5).

	Total cholesterol	HDL cholesterol	TC/HDL ratio	non-HDL cholesterol	LDL cholesterol	LDL/HDL ratio	Triglycerides
Psoriatic patients <i>female</i>	207.73±65.50 (n=11)	60.18±17.55 (n=11)	3.45±0.43 (n=11)	147.55±51.07 (n=11)	132.90±50.90 (n=10)	2.13±0.44 (n=10)	119.82±113.84 (n=11)
<i>male</i>	185.17±30.95 (n=24)	44.13±9.35 (n=23)	4.37±1.08 (n=23)	141.70±31.04 (n=23)	112.09±29.10 (n=22)	2.65±0.87 (n=22)	143.00±60.54 (n=25)
<i>total</i>	192.26±44.98 (n=35)	49.32±14.48 (n=34)	4.08±1.01 (n=34)	143.59±37.95 (n=34)	118.59±37.71 (n=32)	2.49±0.79 (n=32)	135.92±79.58 (n=36)
Controls <i>female</i>	201.71±37.63 (n=653)	61.10±13.88 (n=647)	3.45±0.94 (n=647)	140.86±36.96 (n=647)	119.61±34.03 (n=615)	2.06±0.77 (n=615)	106.54±52.65 (n=642)
<i>male</i>	193.46±38.84 (n=221)	48.63±12.28 (n=216)	4.18±1.17 (n=216)	145.04±38.53 (n=216)	119.57±33.90 (n=204)	2.60±0.92 (n=202)	137.50±86.41 (n=218)
<i>total</i>	199.62±38.09 (n=874)	57.98±14.53 (n=863)	3.63±1.06 (n=863)	141.91±37.38 (n=863)	119.60±33.98 (n=819)	2.19±0.84 (n=817)	114.39±64.32 (n=860)
	p=0.139 p=0.437*	p=0.042 p=0.153*	p=0.008 p=0.383*	p=0.801 p=0.926*	p=0.882 p=0.797*	p=0.047 p=0.566*	p=0.118 p=0.733*

Table 5: means for the different parameters of the lipid profile with standard deviation and number of cases for each subgroup; p-values calculated with t-test or Mann Whitney test according to the distribution of the samples; significance level defined at $p \leq 0.05$; *p-values adjusted for gender and age using linear regression

If the obese population ($BMI \geq 30 \text{ kg/m}^2$) is excluded the results are more promising. It is important to remember that this exclusion criterion could not be met in the psoriatic group because of lacking information. However, since obesity has so many interactions with what is being analyzed, it is felt to be of interest to show the massive change in outcomes.

A significant association was found between lower levels of HDL and psoriasis as well as with higher levels of ACE activity (table 6), after adjustment for age and gender. There is also a strong tendency for an increase in TC/HDL ratio and triglyceride levels in psoriasis, which comes in agreement with many of the basis of this study as well as previous studies [117].

		Total cholesterol	HDL cholesterol	TC/HDL ratio	non-HDL cholesterol	LDL cholesterol	LDL/HDL ratio	Triglycerides	ACE activity
Psoriatic patients	<i>f</i>	207.73±65.50 (n=11)	60.18±17.55 (n=11)	3.45±0.43 (n=11)	147.55±51.07 (n=11)	132.90±50.90 (n=10)	2.13±0.44 (n=10)	119.82±113.84 (n=11)	17.38±7.67 (n=6)
	<i>m</i>	185.17±30.95 (n=24)	44.13±9.35 (n=23)	4.37±1.08 (n=23)	141.70±31.04 (n=23)	112.09±29.10 (n=22)	2.65±0.87 (n=22)	143.00±60.54 (n=25)	19.83±14.66 (n=14)
	<i>t</i>	192.26±44.98 (n=35)	49.32±14.48 (n=34)	4.08±1.01 (n=34)	143.59±37.95 (n=34)	118.59±37.71 (n=32)	2.49±0.79 (n=32)	135.92±79.58 (n=36)	19.09±12.80 (n=20)
Controls	<i>f</i>	200.54±36.89 (n=403)	63.87±14.42 (n=397)	3.27±0.83 (n=397)	137.07±35.39 (n=397)	118.13±33.26 (n=379)	1.95±0.72 (n=379)	96.20±43.07 (n=396)	11.61±6.75 (n=144)
	<i>m</i>	195.77±42.56 (n=130)	51.63±12.79 (n=128)	3.96±1.09 (n=128)	144.17±40.96 (n=128)	122.04±37.08 (n=122)	2.48±0.89 (n=121)	129.20±86.06 (n=128)	13.41±7.97 (n=34)
	<i>t</i>	199.37±38.37 (n=533)	60.89±14.98 (n=525)	3.44±0.95 (n=525)	138.80±36.91 (n=525)	119.09±34.23 (n=501)	2.08±0.80 (n=500)	104.26±58.32 (n=524)	11.95±7.01 (n=178)
		p=0.143 p=0.473*	p<0.001 p=0.011*	p<0.001 p=0.064*	p=0.479 p=0.758*	p=0.943 p=0.873*	p=0.007 p=0.233*	p=0.024 p=0.060*	p=0.023 p=0.003*

Table 6: means for the different parameters of the lipid profile with standard deviation and number of cases for each subgroup when excluded subjects with BMI≥30kg/m²; p-values calculated with t-test or Mann Whitney test according to the distribution of the samples; significance level defined at p≤0,050; *p-values adjusted for gender and age using linear regression; f-female, m-male, t-total

This result reinforces the idea that obesity does have an influence when it comes to assessing the ACE and psoriatic effect on lipid profile and therefore BMI should be calculated in both populations (controls and psoriatic) for a future study. It would also be relevant to inquire the subjects of both groups about their usual medication and exclude or adjust these findings to the intake of statins, other cholesterol lowering agents or any cardiovascular risk altering medication. Diabetes and hypertension are also comorbidities that influence cardiovascular risk and might be associated with psoriasis as discussed above and therefore should be considered in both the psoriatic and control groups and used to adjust statistical results. The clinical information provided regarding the psoriatic group did not include such diagnosis.

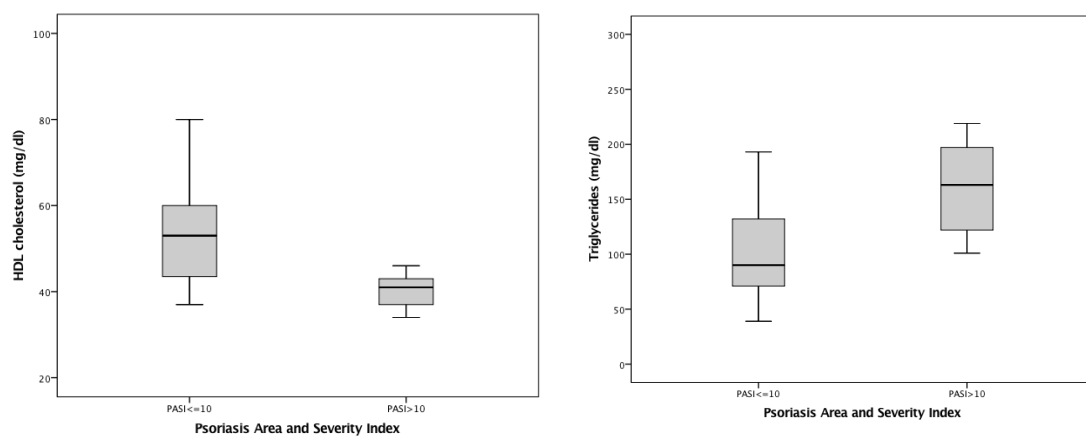


Image 1: on the left, HDL concentrations in psoriatic patients with mild (54.26±14.72) versus moderate to severe disease (41.67±11.04); on the right, triglyceride levels in patients with mild psoriasis (113.38±66.66) compared to patients with moderate to severe disease (163.56±44.95)

In order to estimate an association between psoriasis severity and cardiovascular risk, two subgroups of psoriatic patients were defined, knowing that PASI \leq 10 accounts for a mild psoriasis and PASI $>$ 10 includes both moderate to severe disease [118]. The results in table 7 show a significant association with some of the features in the patient's lipid profile. The HDL level is significantly lower in psoriatic patients with moderate to severe disease when comparing to mild psoriasis (image 1). TC/HDL ratio was found to be higher in patients with a more severe disease, as well as the triglycerides level that is shown to be significantly higher when PASI $>$ 10, all of which when adjusting for age and gender. A tendency was also found for an increase in LDL/HDL ratio with psoriatic severity, after the mentioned adjustment.

	Total cholesterol	HDL cholesterol	TC/HDL ratio	non-HDL cholesterol	LDL cholesterol	LDL/HDL ratio	Triglycerides	ACE activity
PASI \leq10	196.60 \pm 46.20 (n=20)	54.26 \pm 14.72 (n=19)	3.76 \pm 0.89 (n=19)	143.74 \pm 39.51 (n=19)	121.42 \pm 37.41 (n=19)	2.31 \pm 0.69 (n=19)	113.38 \pm 66.66 (n=21)	10.24 \pm 8.22 (n=5)
PASI $>$10	196.44 \pm 38.42 (n=9)	41.67 \pm 11.04 (n=9)	4.88 \pm 1.02 (n=9)	154.78 \pm 32.57 (n=9)	121.13 \pm 36.60 (n=8)	3.01 \pm 0.89 (n=8)	163.56 \pm 44.95 (n=9)	17.20 \pm 11.03 (n=4)
	p=0.814 p=0.604*	p=0.028 p=0.022*	p=0.012 p=0.011*	p=0.279 p=0.228*	p=0.811 p=0.559*	p=0.035 <u>p=0.089*</u>	p=0.021 p=0.020*	p=0.327 p=0.297*

Table 7: means for the different parameters of the lipid profile with standard deviation and number of cases for each subgroup; p-values calculated with t-test or Mann Whitney test according to the distribution of the samples; significance level defined at p \leq 0.05; *p-values adjusted for gender and age

PASI was not found to be significantly correlated with the ACE genotype (one-way ANOVA test p=0.116) with a nonsignificant higher frequency of the II genotype in psoriatic patients with moderate to severe disease (OR=2.25, p=0.338).

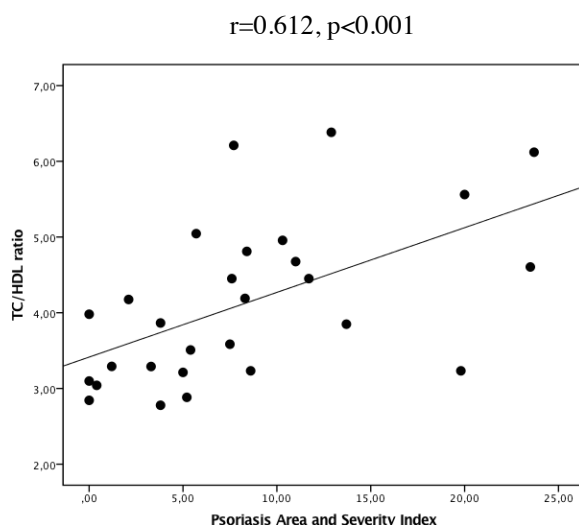


Image 2: graph showing association between TC/HDL ratio and psoriasis severity (estimated by PASI); pearson's correlation; significance level defined at p \leq 0.05

Considering the fact that once PASI is divided into two groups there is some information that is lost in the analysis, the continuous variable was used to check for correlations with the pearson or spearman test (depending on the distribution). This showed that PASI is inversely associated with HDL cholesterol ($r=-0.496$, $p=0.007$) and positively correlated with LDL/HDL ratio ($r=0.437$, $p=0.023$) and triglycerides levels ($r=0.421$, $p=0.020$), indicating a decrease in HDL cholesterol with a concomitant increase in LDL/HDL ratio as well as in triglycerides with psoriasis severity.

As can be seen in image 2, PASI is also strongly correlated to TC/HDL ratio ($r=0.612$, $p<0.001$), which leads to believe that psoriasis severity is in fact associated with an increase in cardiovascular disease as the TC/HDL ratio is one of the lipid indices with higher predictive value [114, 119, 120].

Conclusions

This study found an association between psoriasis and a higher ACE activity level, independent of its genotype. This increase in ACE activity suggest an oxidative stress state that is characteristic of cardiovascular disorders since it is one of the precipitating factors in atherogenesis. The genotype was strongly associated with ACE activity as also found in previous studies, although no association between the ACE I/D polymorphism and this disease or its severity could be demonstrated in our population. It is felt that a larger psoriatic sample with their ACE activities measured would be of importance to assess the true influence of this enzyme in the cardiovascular risk seen in these patients, specially since there appears to be a nonsignificant increase in ACE activity in the population with increased cholesterol levels.

Psoriatic severity was associated with a higher cardiovascular risk estimated by a lower HDL concentrations ($r=-0.496$, $p=0.007$) and higher triglyceride levels ($r=0.421$, $p=0.020$), as well as TC/HDL and LDL/HDL ratios ($r=0.612$, $p<0.001$ and $r=0.437$, $p=0.023$, respectively) which have been shown to have a stronger predictive value for cardiovascular diseases.

Further studies are crucial in order to keep enhancing our knowledge on the overlap of inflammation and psoriasis as a systemic autoimmune chronic disease. In a similar study, it would be important to have more information, with special relevance in using a larger psoriatic population sample and having better information regarding BMI and other comorbidities that alter cardiovascular risk, as mentioned before, as well as medication that could affect our lipid profile levels and underestimate the difference between populations. It would also be of value to have a control group that better represents the normal population, which might not be found within the hospital/clinic environment.

It would also be interesting to assess lipid function and form in psoriatic patients as they may have deeper disturbances in this metabolism than just merely a quantitative issue, as can be perceived in the literature.

Psoriatic patients should be seen as a risk group for cardiovascular disorders and controlled accordingly. New and better markers for severity and for assessing this risk, as ACE might be, may be of worthy pursuit.

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Attachments

Attachment 1:

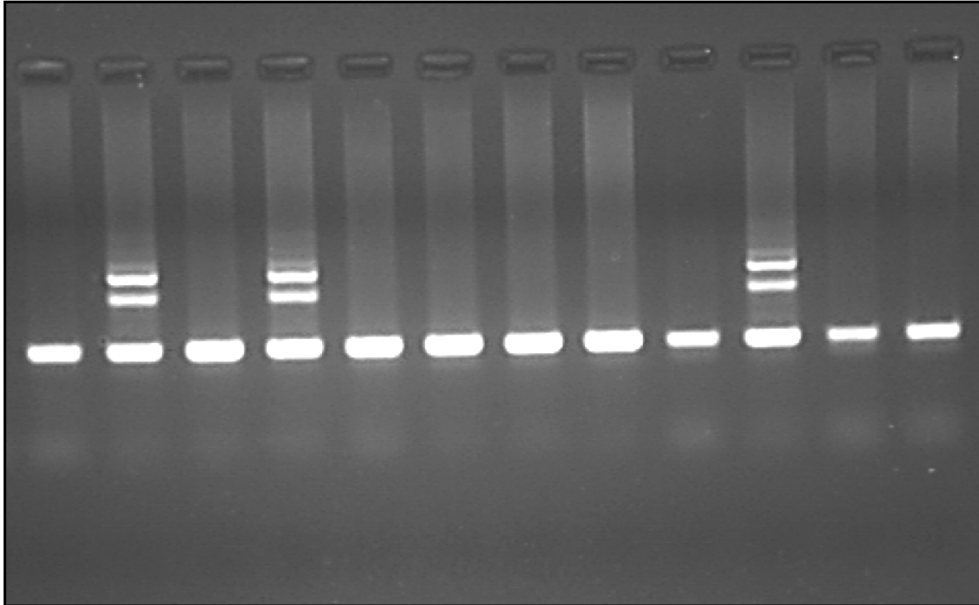


Image 3: electrophoresis gel for the ACE i/d polymorphism; the first column shows a DD phenotype with a fragment of 190bp and the second shows the heterozygous phenotype with the D fragment and the I fragment with 477bp; II genotype not displayed in this set; performed in the lab in May 2015