Association of the Complement System with Subclinical Atherosclerosis in Psoriasis: Findings from an Observational Cohort Study

Laura Mourino-Alvarez[1,2,14], Inés Perales-Sanchez[1,2,14], Emilio Berna-Rico[3], Carlota Abbad-Jaime de Aragon[3], Nerea Corbacho-Alonso[1,2], Tamara Sastre-Oliva[1,2], Cristina Juarez-Alia[1,2], Asunción Ballester-Martinez[3], Maria Castellanos-Gonzalez[4], Mar Llamas-Velasco[5], Pedro Jaen[3], Jorge Solis[6,7,8], Leticia Fernandez-Friera[7,8,9], Neha N. Mehta[10], Joel M. Gelfand[11,12], Maria G. Barderas[1,2,15] and Alvaro Gonzalez-Cantero[3,13,15]

Psoriasis is a chronic and inflammatory disease that affects the skin and joints and is associated with multiple comorbidities and cardiovascular risk factors. Consequently, patients with psoriasis have an increased risk of cardiovascular diseases such as atherosclerosis, a chronic pathology that shares common inflammatory and immune-response mechanisms with psoriasis, including vascular inflammation and complement activation. To better understand the relationship between atherosclerosis and psoriasis, a proteomics study followed by a bioinformatics analysis was carried out, with a subsequent validation step using ELISA and western blotting. When the plasma from patients with psoriasis alone was compared with that from patients with psoriasis and atherosclerosis, 31 proteins of interest related to the complement system and oxygen transport were identified. After the validation phase, 11 proteins appeared to define the presence of subclinical atherosclerosis in patients with psoriasis, indicating the importance of complement cascades in the development of atherosclerotic plaques in individuals with psoriasis. These results are a step forward in understanding the pathological pathways implicated in the cardiovascular risk associated with this population, which may represent an interesting starting point for developing predictive tools that improve the follow-up of these patients and design more effective therapies.

Keywords: Cardiovascular diseases, Inflammation, Mass spectrometry, Proteomics, Vascular biology

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INTRODUCTION

Psoriasis is a complex, chronic, inflammatory disease that is related to immunological processes. Although the skin and joints are the main tissues affected, this pathology is also associated with several comorbidities and cardiovascular (CV) risk factors, such as hypertension and type 2 diabetes. In addition, patients with psoriasis are at increased risk of adverse CV events, including cardiac death, ischemic heart disease, thromboembolism, or arrhythmia (Armstrong et al, 2013; Liu et al, 2022; Raaby et al, 2017). The Framingham risk score estimates the 10-year risk of major adverse CV events in the general population, which includes ischemic stroke, myocardial infarction, and CV-related death. However, it underestimates the long-term risk of patients with chronic inflammatory diseases. Indeed, patients with severe psoriasis may have a risk of major adverse CV events over 10 years 6.2% superior to theoretically calculated with these scores (Mehta et al, 2011). These results demonstrate the difficulty of detecting subclinical atherosclerosis early, making it complicated to take preventive measures to reduce CV

[1]*Department of Vascular Physiopathology, Hospital Nacional de Paraplejicos, SESCAM, Toledo, Spain;* [2]*Department of Vascular Physiopathology, Hospital Nacional de Paraplejicos, IDISCAM, Toledo, Spain;* [3]*Department of Dermatology, Hospital Universitario Ramon y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain;* [4]*Department of Dermatology, Hospital del Sureste, Madrid, Spain;* [5]*Department of Dermatology, Hospital Universitario de la Princesa, Madrid, Spain;* [6]*Department of Cardiology, Hospital Universitario Doce de Octubre, Madrid, Spain;* [7]*Atria Clinic, Madrid, Spain;* [8]*Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain;* [9]*HM Hospitales-Centro Integral de Enfermedades Cardiovasculares HM-CIEC, Madrid, Spain;* [10]*Department of Cardiology, The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, USA;* [11]*Department of Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA;* [12]*Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA; and* [13]*Faculty of Medicine, Universidad Francisco de Vitoria, Madrid, Spain*

[14]*These authors contributed equally to this work.*

[15]*These authors contributed equally as senior authors.*

Correspondence: Alvaro González-Cantero, Department of Dermatology, Hospital Universitario Ramón y Cajal, M-607, km. 9, 100, Madrid 28034, Spain. E-mail: alvarogc261893@hotmail.com and Maria G. Barderas, Laboratorio de Fisiopatologia Vascular, Despacho 3, Edificio de Investigación 1ª planta, Hospital Nacional de Paraplejicos, SESCAM, Toledo 45071, Spain. E-mail: megonzalezb@sescam.jccm.es

Abbreviations: C3, complement 3; CFB, complement factor B; CFD, complement factor D; CFI, complement factor I; CV, cardiovascular; CVD, cardiovascular disease; DPP4, dipeptidyl peptidase-4

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risk in these patients before the onset of any clinical manifestations (González-Cantero et al, 2019).

Atherosclerosis is also a chronic, inflammatory, immune-related disease that is initiated by endothelial dysfunction, lipid deposition in the arterial wall, and infiltration of monocyte-derived macrophages (Teague et al, 2017). Atherosclerosis and psoriasis share several common pathophysiological pathways, such as vascular inflammation, endothelial dysfunction, and oxidative stress–related damage (Manolis et al, 2019; Masson et al, 2020). Furthermore, the proinflammatory cytokine profile of psoriatic plaques is similar to that of vascular atherosclerotic plaques, with a comparable inflammatory infiltrate of T cells, macrophages, and monocytes (Armstrong et al, 2011; Boehncke, 2018; Caiazzo et al, 2018). Importantly, it appears that overactivation of innate and adaptive immune responses is clearly involved in the 2 diseases (Wang et al, 2022). Specifically, it is believed that IL-17/IL-23 axis may be a pivotal factor for comorbid psoriasis and atherosclerosis (Liu et al, 2020). In fact, clinical research shows that optimal control of the inflammation in psoriasis by inhibiting ILs targeting the IL-17/IL-23 axis could subsequently reduce the accompanied atherosclerotic process, although some results are conflicting (Liu et al, 2021; Tsiogka et al, 2023). This controversy may be due to the ambivalent role of IL-17 in atherosclerosis, being both proatherogenic and promoting stable, fibrotic plaques (Allam et al, 2018; Gisterå and Hansson, 2017). An important bridge between the innate immune and the adaptive immune system is the complement pathway, which participates in homeostatic and pathophysiological processes of tissue remodeling as well as the elimination of immune complexes, apoptotic cells, and cellular debris (Ricklin et al, 2010). Different complement factors have been detected in both atherosclerotic (Laine et al, 2002; Speidl et al, 2011) and psoriatic plaques (Takematsu and Tagami, 1992; Uyemura et al, 1993). In fact, these factors may be produced directly within the plaques, which could be related to complement activation (Niculescu and Rus, 2004; Takematsu and Tagami, 1992; Yasojima et al, 2001). Human keratinocytes, the main cell type in the epidermis, are important components of the cutaneous immune system, producing cytokines, chemokines, and some complement proteins such as complement 3 (C3) (Basset-Séguin et al, 1990), complement factor B (CFB) (Yancey et al, 1992), complement factor H (Timár et al, 2006), and terminal complement components (Timár et al, 2007). However, the role of this system is unclear because it has regulatory properties as well as variable roles in the pathogenesis of skin diseases and atherosclerosis (Giang et al, 2018; Kiss and Binder, 2022).

The aim of the study is to delve into the molecular changes that occur during the development of subclinical atherosclerosis in patients with psoriasis to deepen our knowledge about shared mechanisms between psoriatic and atherosclerotic plaque formation (Figure 1). Studying the relationship between the proteome associated with both pathologies may help to obtain new indicators to achieve an early diagnosis as well as improve existing therapies through the definition of potential therapeutic targets.

RESULTS

A total of 62 individuals with psoriasis, classified into 2 groups according to the absence or presence of atherosclerosis, participated in this study: 18 in the discovery phase and 44 in the validation phase. Patients with psoriasis were middle aged and had moderate-to-severe skin disease. The baseline characteristics of the individuals in each group are summarized in Tables 1 and 2.

Quantitative proteomics

By analyzing the proteome of the plasma samples from patients with psoriasis with or without atherosclerosis, 33,457 tandem mass spectrometry spectra corresponding to 355 protein groups were identified. Only the peptides identified as unique were taken into account for the quantitative analysis, and thus, a total of 307 proteins were quantified. Results were represented in a volcano plot (Figure 2a). After the statistical analysis, 31 proteins of interest were identified with a $P < .1$, 30 of which were downregulated in patients with atherosclerosis relative to those without atherosclerosis, and 1 was upregulated (Table 3 and Figure 2b). Consequently, we propose to continue to study these proteins in a larger cohort of 44 patients to determine their significance in this disease.

Protein functional annotation

We performed a functional annotation analysis of the proteins differentially expressed between the 2 study groups, which resulted in 3 clusters (Table 4). Cluster 1 was formed by 5 proteins related to oxygen transport and binding, whereas the second cluster was made up of 10 proteins related to complement activation and the immune response. The final cluster was made up of 6 proteins related to serin-type endopeptidase activity. In addition, proteins were grouped into 23 biological processes gene ontology terms (Figure 2c). Most proteins were grouped in complement-related terms.

Validation of the proteins of interest

To validate the results of the proteomics studies, we used 2 orthogonal techniques: western blotting and ELISA. We analyzed the C3, C4BPB, CFB, complement factor D (CFD), and complement factor I (CFI) proteins in western blots, which confirmed the weaker expression of these proteins in patients with psoriasis and atherosclerosis relative to that in patients with psoriasis alone ($P = .0001$, $P = .008$, $P = .013$, $P = .017$, and $P = .047$, respectively) (Figure 3a–e). In addition, ELISA assays confirmed the lower levels of HBA, HBB, C6, C8a, and dipeptidyl peptidase-4 (DPP4) in the plasma from patients with psoriasis and atherosclerosis relative to the levels in the plasma from patients with psoriasis alone ($P = .049$, $P = .035$, $P = .021$, $P = .015$, and $P = .005$, respectively) (Figure 4a–e). By contrast, CFHR2 was upregulated in patients with psoriasis with atherosclerosis ($P = .006$) (Figure 4f).

For analyzing the sensitivity and specificity of the different proteins, receiver operating characteristic curves were calculated. For each technique, the diagnostic power of all the proteins as a panel was much better than that of the proteins alone, indicating that these panels of proteins could discriminate patients with and without atherosclerosis

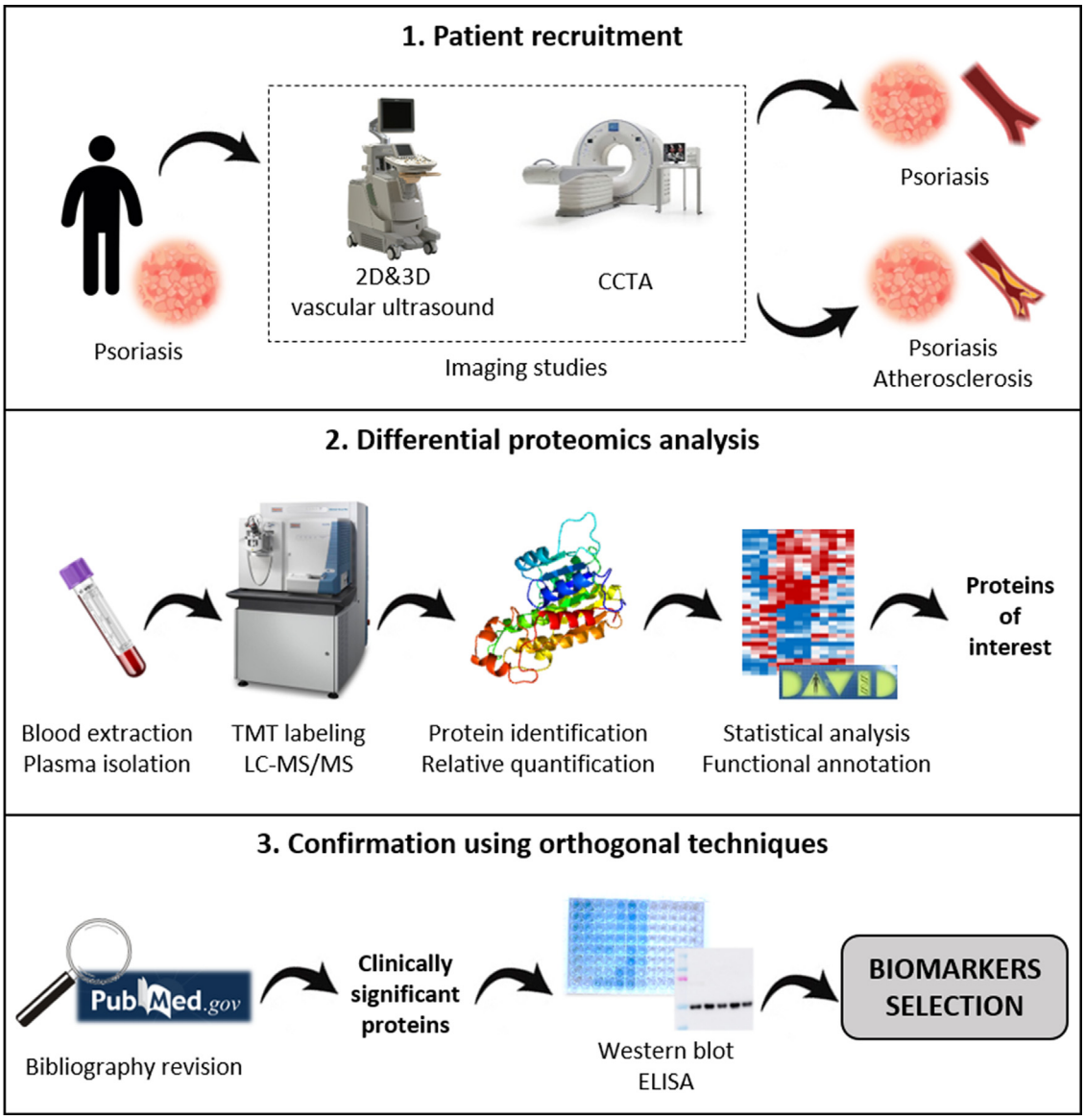

Figure 1. Overview of the experimental workflow. Blood was collected from patients with psoriasis, classified into 2 groups according to the absence/presence of atherosclerosis. Plasma samples were labeled with TMT tags and analyzed using LC-MS/MS. After statistical analysis and functional annotation, proteins of interest were selected for confirmation using orthogonal techniques. 2D, 2-dimensional; 3D, 3-dimensional; CCTA, coronary computed tomography angiography; LC-MS/MS, liquid chromatography–tandem mass spectrometry; TMT, tandem mass tags.

(western blot area under the curve = 0.91, $P < .001$ [Figure 3f–g]; ELISA area under the curve = 1.00, $P = .002$ [Figure 4g–h]). The discriminating power of these panels has been better than that of other traditional CV risk scores and markers, such as the Pooled Cohort Equation, Framingham risk score, or CRP high sensitivity as well as promising biomarkers such as neutrophil to lymphocyte ratio receiver operating characteristic curves are shown in the Supplementary Figure S1).

DISCUSSION

This study focuses on the search for molecular markers of subclinical atherosclerosis, that is, during the asymptomatic period, in patients with psoriasis. The life expectancy of patients with psoriasis is reduced by 4–5 years owing to CV diseases (CVDs), and they are at greater risk of myocardial infarction at a younger age than individuals without psoriasis (Abuabara et al, 2010; Gelfand et al, 2006). Nevertheless, the mechanisms underlying early coronary artery disease associated with psoriasis are not well-understood. In this context, there is an urgent need to discover markers that are of practical value for the early identification of patients with psoriasis who are at higher risk of developing CVDs, most of whom would benefit from prompt therapeutic intervention (Ogdie et al, 2015). In this study, we set out to define new indicators of subclinical atherosclerosis in patients with psoriasis to help improve their risk stratification and clinical management with more personalized treatments. This is supported by the results of the receiver operating characteristic curves, in which sensitivity and specificity have been evaluated. As such, the protein profiles of plasma from patients with psoriasis were compared according to the

Table 1. Clinical Characteristics of the Subjects Used in the Discovery Phase

Markers	Psoriasis (n = 9)	Psoriasis + Atherosclerosis (n = 9)	*P*-Value
Clinical characteristics			
Age, y	45.44 ± 3.94	49.89 ± 5.51	0.07
Sex, % M/F	78/22	78/22	1.00
BMI, Kg/m^2	33.57 ± 8.74	31.19 ± 3.82	0.46
Waist circumference, cm	110.44 ± 18.66	106.889 ± 10.89	0.63
Systolic pressure, mmHg	131.38 ± 10.57	138.11 ± 8.313	0.17
Diastolic pressure, mmHg	88.75 ± 12.95	93.78 ± 8.35	0.37
Hypertension, %	33	44	0.73
Dyslipidemia, %	33	56	0.44
Diabetes, %	0	0	1.00
Smoking, %	22	22	1.00
Psoriasis characteristics			
PSO start date, age	23.33 ± 11.16	28.44 ± 11.09	0.34
PSO duration, y	21.56 ± 12.30	20.89 ± 10.39	0.90
PASI score	12.44 ± 7.01	7.61 ± 6.06	0.14
BSA, %	14.11 ± 9.07	6.78 ± 5.24	0.05
Psoriatic arthritis, %	22	56	0.26
Treatment history			
Biologics, %	22	33	0.73
Nonbiological systemic treatment, %	56	89	0.26
Laboratory values			
Glucose, mg/dl	94.11 ± 19.81	93.44 ± 10.39	0.93
Calcium, mg/dl	9.156 ± 0.44	9.422 ± 0.36	0.18
ALT, U/l	36.78 ± 24.28	34.56 ± 33.186	0.87
AST, U/l	25.78 ± 10.06	24.44 ± 12.65	0.81
Insulin, μU/ml	14.34 ± 5.82	10.70 ± 4.50	0.17
Lipid profile			
Triglycerides, mg/dl	150.56 ± 91.21	123.33 ± 35.12	0.42
Total cholesterol, mg/dl	196.44 ± 30.61	212.44 ± 35.06	0.32
HDL cholesterol, mg/dl	45.11 ± 11.41	49.78 ± 11.63	0.40
LDL cholesterol, mg/dl	123.22 ± 22.93	137.56 ± 28.69	0.26
CV risk assessment			
hsCRP	3.10 ± 1.63	4.99 ± 3.12	0.21
NLR	2.21 ± 0.90	2.02 ± 0.86	0.66
ASCVD-R	3.02 ± 2.18	5.08 ± 3.59	0.05
FRS	6.37 ± 2.48	10.37 ± 5.00	0.16

Abbreviations: ALT, alanine aminotransferase; ASCVD-R, atherosclerotic cardiovascular disease risk calculated using pooled cohort equation; AST, aspartate aminotransferase; BMI, body mass index; BSA, body surface area; CV, cardiovascular; FRS, Framingham risk score; HDL, high-density lipoprotein; hsCRP, high-sensitivity CRP, LDL, low-density lipoprotein; M/F, male/female; NLR, neutrophil-to-lymphocyte ratio; PSO, psoriasis.

presence/absence of subclinical atherosclerosis. Consequently, 11 proteins that appeared to define the presence of subclinical atherosclerosis in patients with psoriasis were validated in a further patient cohort. These proteins can be grouped into 3 clusters related to their activities: oxygen binding/transport, complement pathway/activation, and the immune response.

Hemoglobin is an iron-rich protein that binds and carries oxygen throughout the body. In addition, it also transports nitric oxide, which regulates blood vessel tension. This protein is made up of 4 polypeptide chains: 2 alpha and 2 beta chains that join together closely. In this work, we found lower levels of these chains in patients with psoriasis and atherosclerosis. A low blood hemoglobin concentration can lead to serious CV complications (Kuhn and Diederich, 2017), and it has been associated with negative health outcomes in patients with CVD, related to a high risk of thromboembolism, high-output cardiac failure, left ventricular hypertrophy, and a possible proatherogenic status (Anderson et al, 2009). The regulation of blood vessel tension through the transport of nitric oxide by hemoglobin is fundamental because nitric oxide regulates blood vessel vasodilation, but it also mediates platelet inhibition, insulin sensitivity, and inflammatory reactions (Conti et al, 2004; Gwozdzinski et al, 2021). The low HBA and HBB levels associated with higher CV risk in patients with psoriasis indicate that red blood cells may play an important role in the development of atherosclerosis in these patients, possibly also influenced by altered nitric oxide bioavailability.

Most proteomic differences we found are related to the complement pathway, with surprisingly lower levels of C3, CFI, C4BPB, CFB, CFD, complement 6, complement 8a, and DPP4 in the group of patients with psoriasis and atherosclerosis than in patients without atherosclerosis and higher

Table 2. Clinical Characteristics of the Subjects Used in the Validation Phase

Markers	Psoriasis (n = 21)	Psoriasis + Atherosclerosis (n = 23)	*P*-Value
Clinical characteristics			
Age, y	44.00 ± 6.58	51.82 ± 6.11	0.00
Sex, % M/F	38/62	17/83	0.13
BMI, Kg/m^2	31.54 ± 7.40	31.35 ± 6.18	0.92
Waist circumference, cm	105.81 ± 18.343	108.83 ± 11.88	0.52
Systolic pressure, mmHg	130.47 ± 14.57	132.91 ± 8.59	0.50
Diastolic pressure, mmHg	89.67 ± 11.18	87.30 ± 10.98	0.48
Hypertension, %	33	52	0.21
Dyslipidemia, %	19	43	0.09
Diabetes, %	0	13	0.09
Smoking, %	43	43	0.97
Psoriasis characteristics			
PSO start date, age	22.19 ± 11.87	27.38 ± 17.15	0.34
PSO duration, y	18.14 ± 12.22	17.07 ± 11.51	0.80
PASI score	10.67 ± 6.81	6.77 ± 4.11	0.03
BSA, %	12.17 ± 9.48	7.70 ± 5.04	0.06
Psoriatic arthritis, %	5	26	0.06
Treatment history			
Biologics, %	38	14	0.08
Nonbiological systemic treatment, %	80	82	0.88
Laboratory values			
Glucose, mg/dl	85.17 ± 24.72	97.65 ± 20.74	0.08
Calcium, mg/dl	9.15 ± 0.45	9.44 ± 0.37	0.03
ALT, U/l	33.67 ± 29.03	97.65 ± 20.74	0.86
AST, U/l	24.29 ± 10.37	26.09 ± 13.81	0.63
Insulin, μU/ml	13.15 ± 7.93	11.20 ± 3.76	0.33
HOMA-IR	50.55 ± 40.94	42.56 ± 24.43	0.43
Lipid profile			
Triglycerides, mg/dl	152.24 ± 124.74	125.43 ± 73.26	0.39
Total cholesterol, mg/dl	196.52 ± 43.03	199.78 ± 33.43	0.78
HDL cholesterol, mg/dl	55.86 ± 43.92	47.48 ± 11.39	0.38
LDL cholesterol, mg/dl	125.52 ± 29.30	128.13 ± 28.71	0.77
CV risk assessment			
hsCRP	3.20 ± 3.70	4.84 ± 3.46	0.20
NLR	2.25 ± 0.85	2.49 ± 1.12	0.44
ASCVD-R	4.25 ± 4.08	4.93 ± 4.28	0.59
FRS	7.86 ± 6.99	10.67 ± 7.52	0.21

Abbreviations: ALT, alanine aminotransferase; ASCVD-R, atherosclerotic cardiovascular disease risk calculated using pooled cohort equations; AST, aspartate aminotransferase; BMI, body mass index; BSA, body surface area; CV, cardiovascular; FRS, Framingham risk score; HDL, high-density lipoprotein; hsCRP, high-sensitivity CRP; LDL, low-density lipoprotein; NLR, neutrophil-to-lymphocyte ratio; M/F, male/female; PSO, Psoriasis.

levels of CFHR2 (Figure 5). The complement system is one of the cornerstones of innate immunity, and it is composed of >30 soluble plasma and membrane proteins. Different stimuli can activate the system through 3 distinct protease cascades: the classical, lectin, and alternative pathways. All of these revolve around the proteolytic cleavage of the central C3 complement component, which serves as a convergence point for further downstream effector functions. Importantly, the role of complement in atherogenesis is complicated because its activation has dual effects: depending on the stage of complement activation, it can mitigate or promote lesion formation (Kiss and Binder, 2022; Speidl et al, 2011). On the one hand, the formation of anaphylatoxins through the activation of the cascade complex can induce proinflammatory signaling, which has proatherogenic effects and may promote plaque vulnerability (Lewis et al, 2010; Li et al, 2013). On the other hand, complement initiation resulting in the cleavage of the central complement component C3 appears to have a crucial role in tempering lesion progression by aiding the clearance of apoptotic cells (Bhatia et al, 2007; Wei et al, 2020).

This way, the complement system is a double-edged sword that must be tightly regulated through membrane-bound and fluid phase regulators to maintain its housekeeping functions. Among the fluid phase regulators, CFI plays a key role in regulating the 3 complement cascades by inhibiting activated C3b and C4b proteins (Kiss and Binder, 2022; Nilsson et al, 2011; Noris and Remuzzi, 2013). Significantly, C4BPB is a cofactor of CFI that regulates the classical pathway. We found these inhibitors to be downregulated in the plasma from patients with psoriasis and atherosclerosis, possibly indicating activation of the complement system. In this sense, the lower levels of C3 in the plasma of patients with atherosclerosis could reflect the cleavage of this protein through

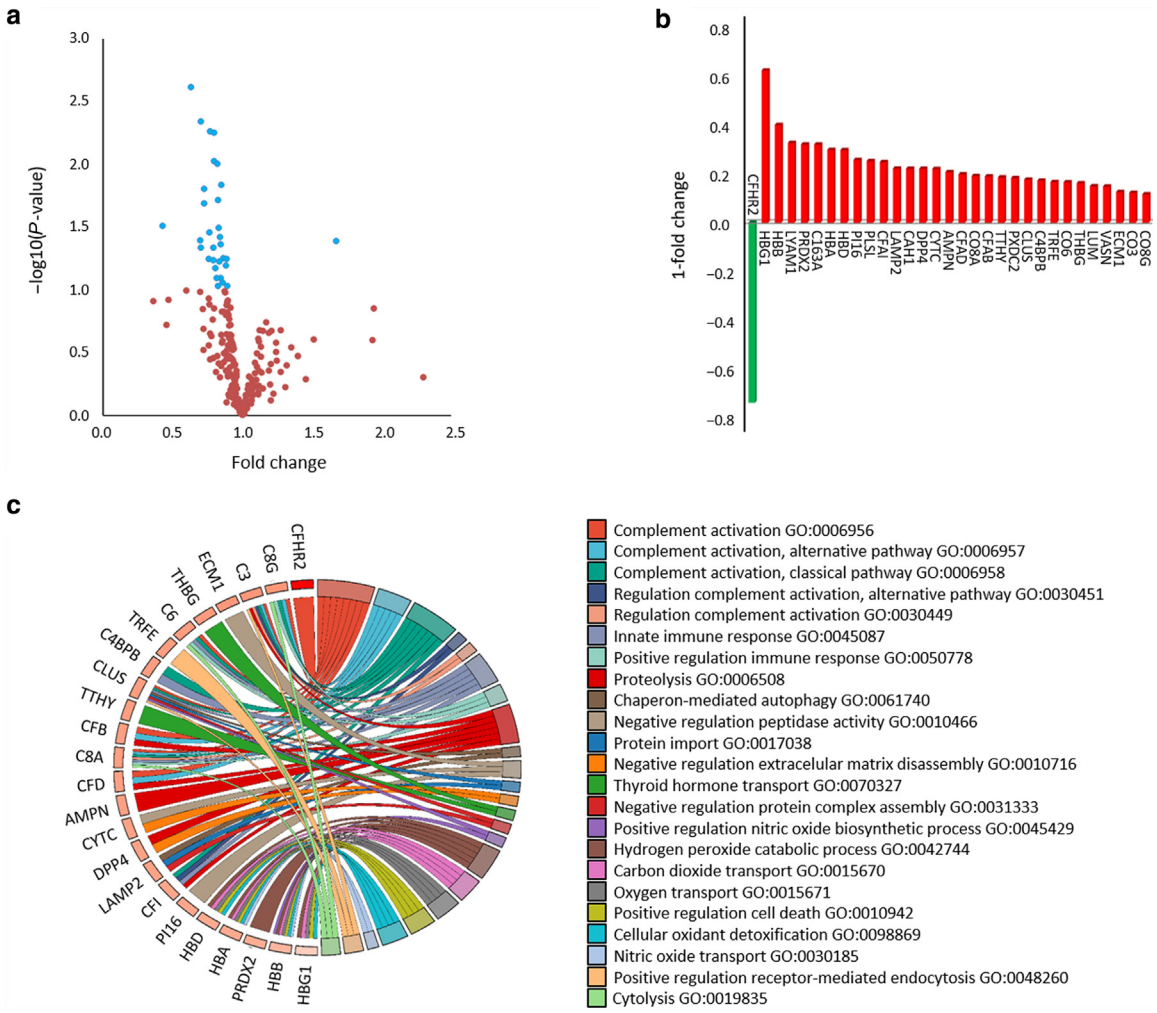

Figure 2. Statistical analysis and functional annotation of proteins of interest. (**a**) Volcano plot. Proteins of interest are shown in blue. (**b**) Proteins of interest found in the discovery phase. Proteins with lower expression levels in patients with atherosclerosis are shown in red, whereas proteins with higher expression levels are shown in green. (**c**) Chord diagram showing the GO of the 31 proteins of interest. The diagrams show the clustered genes and their assigned GO terms, which are connected by ribbons. On the right side of the chord plot, different colors represent different GO terms. GO, gene ontology.

complement activation. Nevertheless, previous studies on plasma C3 levels and CV risk factors gave contradictory results, and although circulating levels were positively correlated with conventional CV risk factors and coronary artery disease in some studies (Ajjan et al, 2005; Muscari et al, 1998; Széplaki et al, 2004), elsewhere, only complement component C4 but not C3 levels were associated with CVD (Cavusoglu et al, 2007; Engström et al, 2007). These discrepancies may be due to the different degrees of C3 activation in individuals from different cohorts, with different characteristics. In this study, we assessed a cohort of patients with psoriasis, and the results obtained indicate a possible activation of the classical pathway rather than the alternative pathway during atherogenesis in this type of patient. In support of this hypothesis, we found lower CFB and CFD levels, which are specific to alternative activation. Importantly, we also found higher levels of CFHR2 in patients with psoriasis with atherosclerosis, a protein that inhibits C3 convertase activity such that the amplification loop of the alternative pathway may be inhibited in these patients (Skerka et al, 2013).

Continuing with the complement pathways, all 3 cascades converge in the generation of the terminal membrane attack complex (MA, C5b-9), made up of the C5b, C6, C7, C8, and several C9 proteins. The formation of this complex explains the lower levels of free C6 and C8a that were found in patients with psoriasis and atherosclerosis. Furthermore, C5b-9 is an independent risk factor for stroke and unstable carotid plaques, and it could serve as a biomarker to predict the severity and outcome as well as carotid plaque stability in patients with stroke (Si et al, 2019). In terms of CFHR2, a study in children with chronic kidney disease highlighted a positive correlation between CFHR2 levels and left ventricular mass and hypertension, indicating that this protein may participate in chronic kidney disease pathogenesis (Liao et al, 2022). DPP4 or CD26 is a ubiquitously expressed serine protease that circulates in a monomeric soluble form but also exists in a homodimeric transmembrane form. DPP4 is widely expressed in the vascular system, indicating that it may be involved in the onset and progression of CVD (Chen et al, 2022). With regard to the complement pathway, DPP4 may

Table 3. List of Proteins of Interest

Abbreviation	Uniprot Accession	Protein Names	Ratio PSO+AT/PSO	*P*-Value
HBB	P68871	Hemoglobin subunit beta	0.63	0.002
PRDX2	P32119	Peroxiredoxin-2	0.71	0.005
CFI	**P05156**	**Complement factor I**	**0.77**	**0.005**
CYTC	P01034	Cystatin-C	0.80	0.006
DPP4	**P27487**	**Dipeptidyl peptidase 4**	**0.80**	**0.010**
C8A	**P07357**	**Complement component C8 alpha chain**	**0.82**	**0.010**
THBG	P05543	Thyroxine-binding globulin	0.85	0.015
HBA	**P69905**	**Hemoglobin subunit alpha**	**0.73**	**0.016**
CFB	**P00751**	**Complement factor B**	**0.83**	**0.019**
HBD	P02042	Hemoglobin subunit delta	0.73	0.021
HBG1	P69891	Hemoglobin subunit gamma-1	0.43	0.031
PXDC2	Q6UX71	Plexin domain-containing protein 2	0.83	0.032
PLSL	P13796	Plastin-2	0.77	0.035
C4BPB	**P20851**	**C4b-binding protein beta chain**	**0.84**	**0.038**
LYAM1	P14151	L-selectin	0.70	0.041
CFHR2	**P36980**	**Complement factor H-related protein 2**	**1.68**	**0.041**
C6	**P13671**	**Complement component C6**	**0.85**	**0.044**
CAH1	P00915	Carbonic anhydrase 1	0.80	0.046
C163A	Q86VB7	Scavenger receptor cysteine-rich type 1 protein M130	0.71	0.046
VASN	Q6EMK4	Vasorin	0.86	0.057
C3	**P01024**	**Complement C3**	**0.89**	**0.057**
PI16	Q6UXB8	Peptidase inhibitor 16	0.76	0.057
LAMP2	P13473	Lysosome-associated membrane glycoprotein 2	0.80	0.058
CLUS	P10909	Clusterin	0.84	0.060
ECM1	Q16610	Extracellular matrix protein 1	0.88	0.064
AMPN	P15144	Aminopeptidase N	0.81	0.068
TRFE	P02787	Serotransferrin	0.85	0.081
CFD	**P00746**	**Complement factor D**	**0.82**	**0.081**
LUM	P51884	Lumican	0.86	0.088
C8G	P07360	Complement component C8 gamma chain	0.89	0.093
TTHY	P02766	Transthyretin	0.83	0.094

Abbreviation: DPP4, dipeptidyl peptidase-4.

PSO+AT denotes patients with psoriasis and atherosclerosis, and PSO denotes patients with only psoriasis. This table shows abbreviations, accession numbers according to Uniprot, protein name, and statistical results (*P*-value according to Student *t*-test and ratio PSO+AT/PSO). The ratio indicates the fold change between the 2 groups of the study. It indicates downregulation of the protein in patients with PSO+AT when the ratio is <1 and an upregulation when ratio is >1. Validated proteins are shown in bold.

be related to activation of the complement system through the lectin cascade because DPP4 inhibitors attenuate this activation (Hoffmann-Petersen et al, 2021). In psoriatic plaques, DPP4 is expressed by keratinocytes, fibroblasts, mast cells, and lymphocyte T (both CD4+ and CD8+) (Patel et al, 2021). Moreover, different psoriasis treatments may alter DPP4 activity (Bonnekoh et al, 2007; Kongthong et al, 2019; Raynaud et al, 1992; Yldrm et al, 2011). DPP4 inhibitors are now widely used to treat type 2 diabetes mellitus in adults, and they are being studied in several other ongoing phase III trials. Although metabolic function seems to be clearly improved by these treatments, the effects of long-term DPP4 inhibition on the immune system and on regulatory processes such as T-cell maturation and activation are not fully understood. It is therefore important to further study this protein in the context of psoriasis and atherosclerosis to clarify whether its downregulation is correlated with reduced DDP4 enzymatic activity. In addition, it will be of interest to better understand the role of complement and the immunological system in these pathologies when they coexist in the same patient.

Our data point to the complement system as one of the main effectors in the development of atherosclerosis in patients with psoriasis. Dysregulation of this system may be responsible for the increased CV risk in patients with psoriasis, especially considering the ability of keratinocytes to produce components of this pathway. It seems clear that understanding the activation of this complex system is essential to better understand the link between psoriasis and atherosclerosis.

This work has certain limitations, such as the number of patients recruited for the study. In the future, these results should be validated in a much larger cohort to confirm the clinical utility of the proteins described in this study. On the other hand, the objective of this work was to carry out a comparative proteomic study between patients with psoriasis with subclinical atherosclerosis and those without. From the results obtained, we believe that a specific study of the 3 complement pathways would be necessary, including a functional analysis of the enzymes involved. We also have a statistical limitation during the selection of proteins of interest after quantitative proteomic analysis. We raised the *P*-value

Table 4. Functional Analysis of the Proteins of Interest

Clusters	Function	Number of Proteins	Enrichment Score	Abbreviated Names of Proteins
Cluster 1	Oxygen/nitrogen transport and binding	5	6.23	HBA, HBB, HBD, HBG1, PRDX2
Cluster 2	Complement activation and immune response	10	5.36	CLU, C3, C6, C8A, C8G, C4BPB, CFB, CFD, CFHR2, CFI
Cluster 3	Complement pathway	6	2.48	AMPN, C3, CFB, CFD, CFI, DPP4

Abbreviations: AMPN, aminopeptidase N; C3, complement 3; C4BPB, C4b-binding protein beta chain; C6, complement 6; C8A, complement component C8 alpha chain; C8G, complement component C8 gamma chain, CFB, complement factor B; CFD, complement factor D; CFHR2, complement factor H-related protein 2; CFI, complement factor I; CLU, clusterin; DPP4, dipeptidyl peptidase-4; HBA, hemoglobin subunit alpha; HBB, hemoglobin subunit beta; HBD, hemoglobin subunit delta; HBG1, hemoglobin subunit gamma-1, PRDX2, peroxiredoxin-2.

The proteins identified are represented in clusters according to their function, showing the number of proteins and the enrichment score of each cluster as well as the abbreviated name of each protein.

of the selected proteins with the aim of assessing whether any of the proteins with a *P*-value slightly higher than .05 were of biological interest, especially because we found that some of them had a close functional relationship with proteins that were significantly different. Nevertheless, in the subsequent phase, only those with a $P < .05$ were considered validated. However, in the initial phase of the study, we did not want to focus on the statistical cutoff but rather on the physiological significance of the proteins.

In summary, our results indicate an important role of complement cascades in the development of subclinical atherosclerosis in patients with psoriasis. Improving the

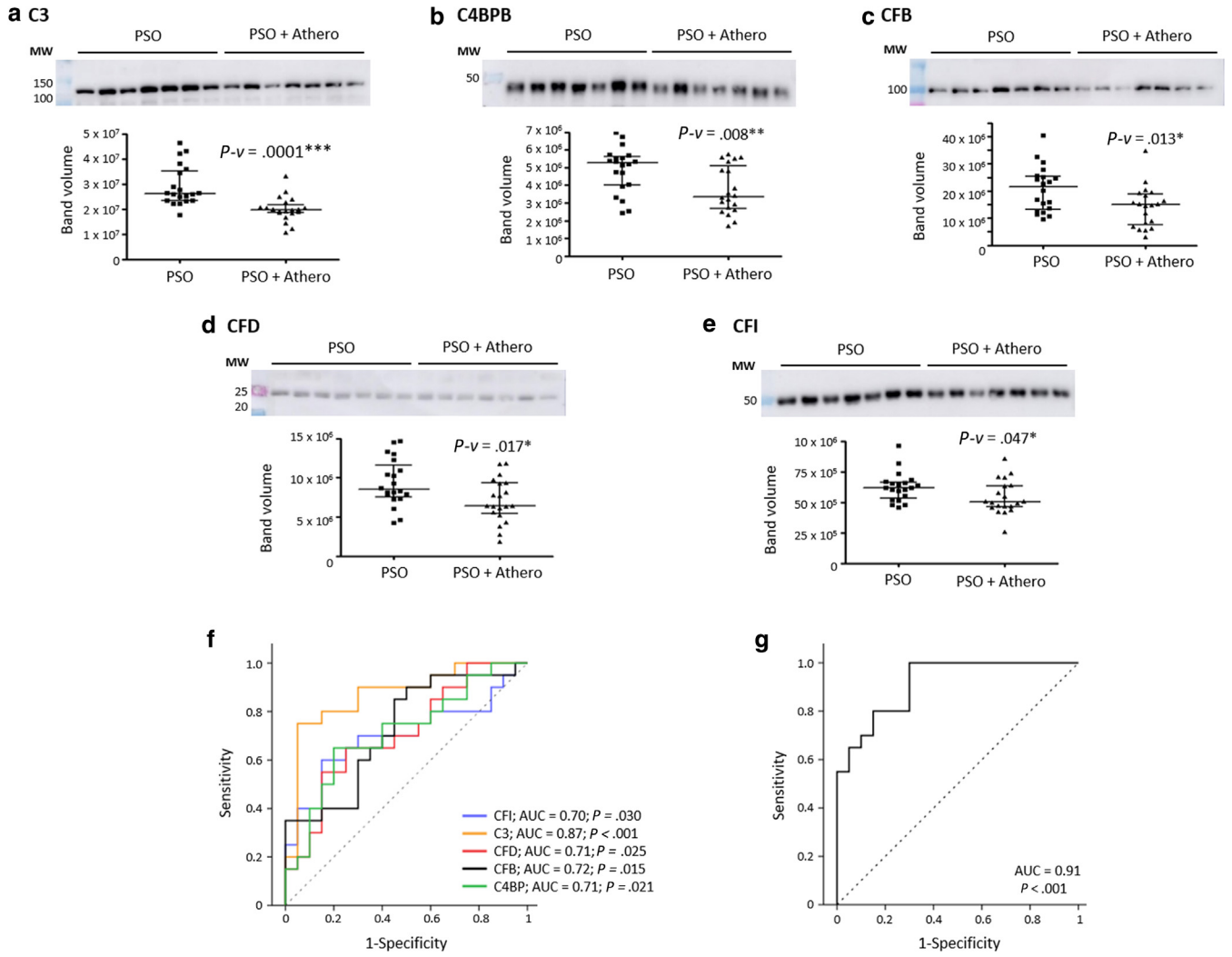

Figure 3. Confirmation of the differentially expressed proteins using western blot. (**a**) C3, (**b**) C4BPB, (**c**) CFB, (**d**) CFD, and (**e**) CFI showed statistical differences between the 2 groups of study. (**f**) ROC curves of individual proteins and (**g**) as a panel. The AUC and *P*-values are shown. *$P < .05$, **$P < .01$, and ***$P < .001$. athero, atherosclerosis; AUC, area under the curve; CFB, complement factor B; CFD, complement factor D; CFI, complement factor I; PSO, psoriasis; ROC, receiver operating characteristic.

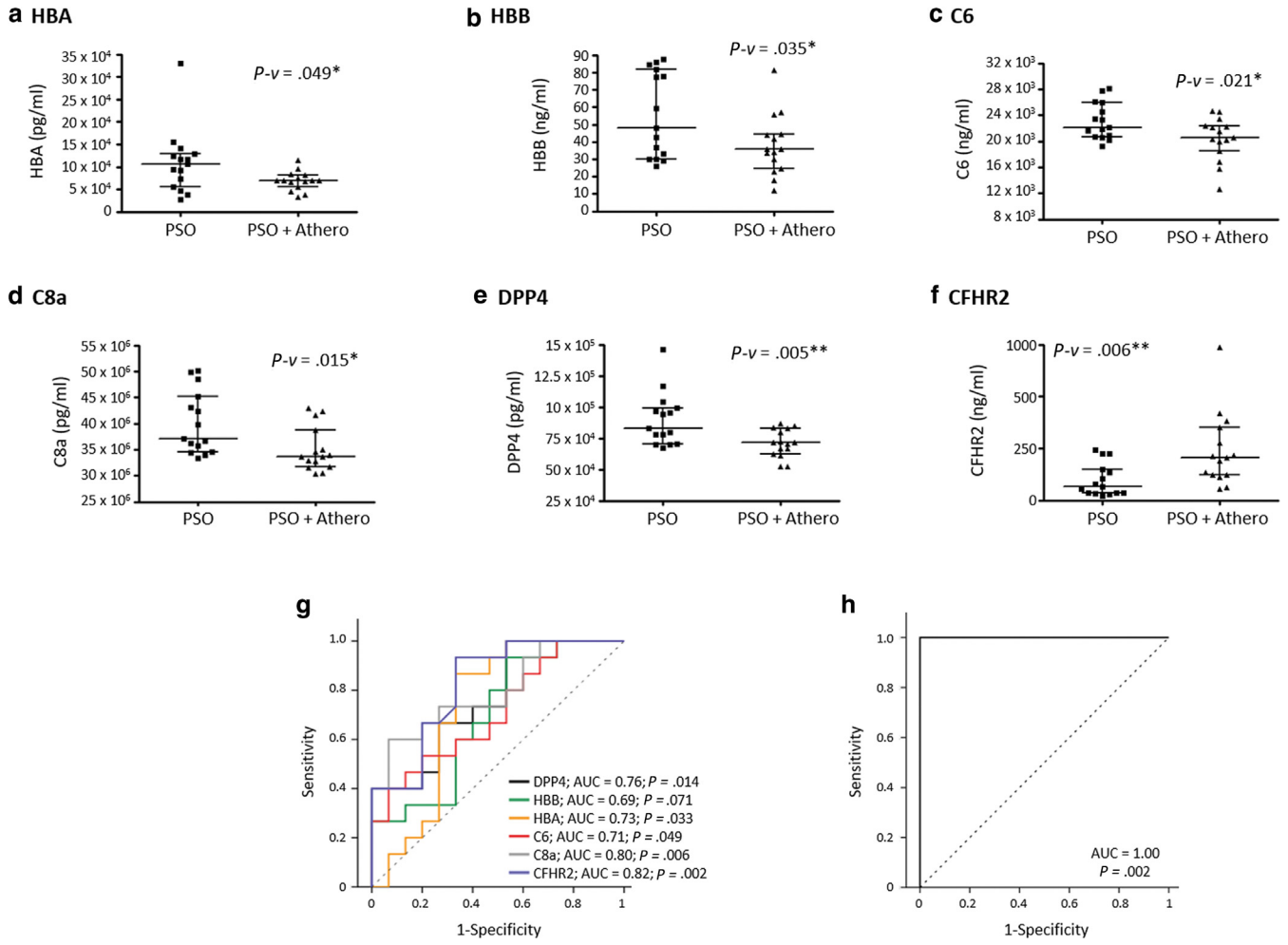

Figure 4. Confirmation of the differentially expressed proteins using ELISA. (**a**) HBA, (**b**) HBB, (**c**) C6, (**d**) C8a, and (**e**) DPP4 are decreased in the group with psoriasis and athero, (**f**) whereas CFHR2 is increased. (**g**) ROC curves of individual proteins and (**h**) as a panel. The AUC and *P*-values are shown. $*P < .05$, $**P < .01$, and $***P < .001$. athero, atherosclerosis; AUC, area under the curve; C6, complement 6; DPP4, dipeptidyl peptidase-4; PSO, psoriasis; ROC, receiver operating characteristic.

understanding of the specific molecular mechanisms involved in this condition will be essential to shift toward personalized medicine for this specific group of patients. On the one hand, it is important to define the pathophysiological pathways that cause patients with psoriasis to be at a higher risk of CVD. This would allow us to obtain an early diagnosis of CVD as well as to predict which patients need a closer medical follow-up. In addition, these studies could lead to the development of more effective therapies for these patients, reducing their mortality and improving their QOL.

MATERIALS AND METHODS

Patient selection

This study included patients from the EDSAP (Early Detection and Progression of Subclinical Atherosclerosis in Psoriasis) study, an observational, longitudinal, prospective cohort of patients with psoriasis who were voluntarily recruited at the dermatology consultations of the Hospital Ramón y Cajal in Madrid. All patients with psoriasis were aged between 30 and 65 years, they had been diagnosed clinically with psoriasis by an expert dermatologist, and they were considered by the investigator to be candidates for biological therapy. Only patients without systemic psoriasis treatments for at least 4 weeks before the study (3 months in the case of anti–IL-23 agents) were included. Patients were excluded if they had a history of CVD (myocardial infarction, angina pectoris, peripheral vascular disease, aortic aneurysm, angioplasty, cardiac surgery, atrial fibrillation, or any other cardiological pathology), as were patients who were receiving any oncological treatment; those with a history of transplantation receiving active immunosuppressor or immunomodulatory therapy; and patients with morbid obesity (body mass index $\geq$40 kg/m^2), chronic liver disease (except for nonalcoholic fatty liver disease), oncological disease, chronic kidney disease (glomerular filtration rate <60 ml/min per 1.73 m^2), any other chronic inflammatory disease, the presence of any pathology that decreases life expectancy to less than 3 years, or any disease or condition that may affect adherence to the study protocol. In addition, participants who had undergone a thoracic computerized tomography scan in the previous year or who were pregnant or breastfeeding were also excluded.

The study protocol was approved by the Ethics Committee at the Hospital Ramón y Cajal (Madrid, Spain). All patients signed an informed consent before their inclusion in the study.

At baseline, each patient was assessed for the presence or absence of subclinical atherosclerosis using different noninvasive

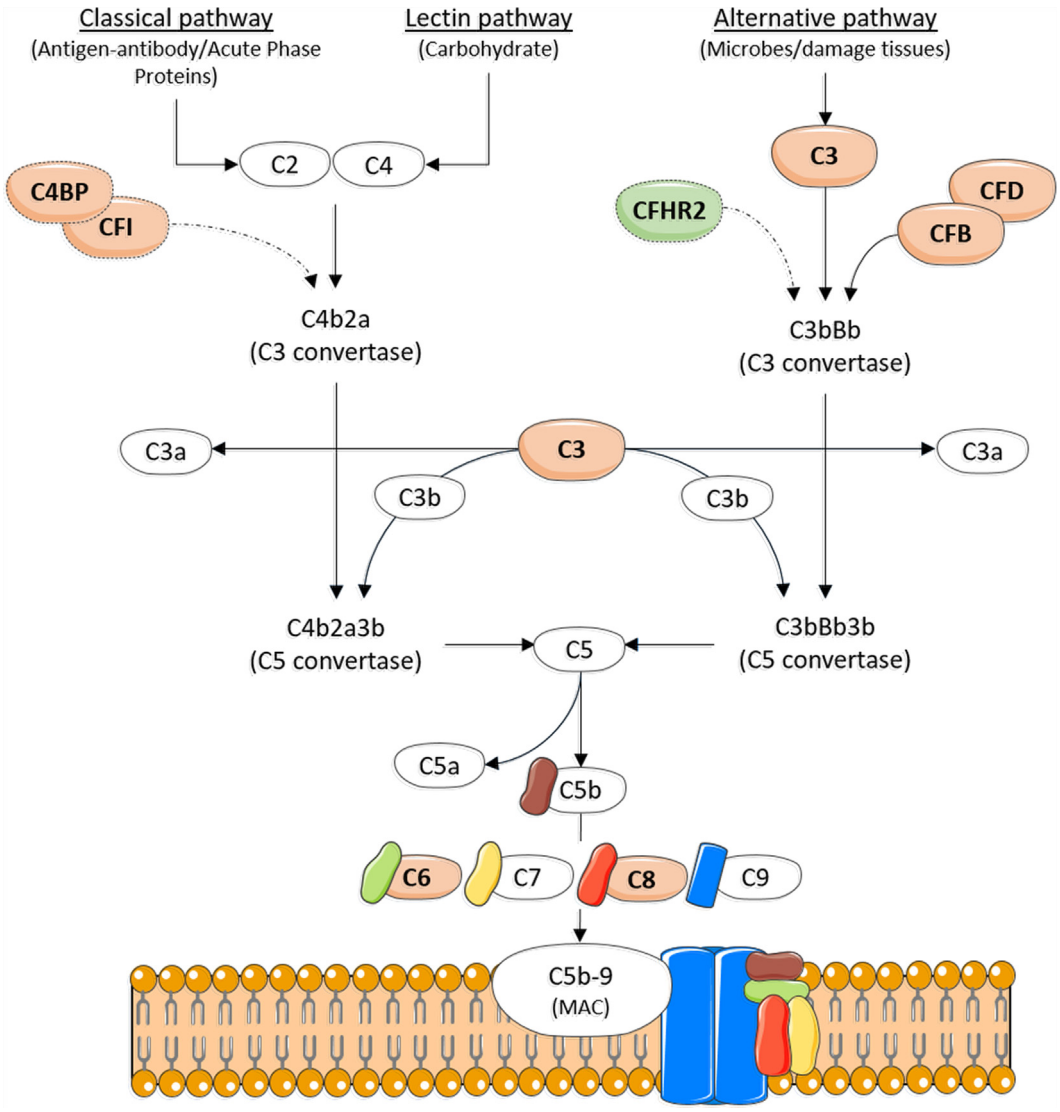

Figure 5. Scheme of the 3 cascades that initiate the complement system. Classical pathway activation occurs after binding of component C1q to antibody–antigen complexes or certain acute phase proteins, whereas the lectin pathway is activated through the interaction with carbohydrate structures predominantly present on invading pathogens. After cleavage of C4 and C2, C3 convertase (C4b2a) is formed. C3 convertase generates C3b and the anaphylatoxin C3a through cleavage of native C3. In contrast to the classical and lectin pathways, the alternative pathway is activated by low-grade spontaneous hydrolysis of systemic native C3. Hydrolyzed C3 can be associated with CFB, which is subsequently activated by CFD. When C5 convertase is formed through cleavage, it initiates the terminal complement cascade. C5 convertases cleave C5 into C5b and anaphylatoxin C5a, eventually resulting in the assembly of C5b-9 by combining C5b with C6-C9. C3, complement 3; C5, complement 5; CFB, complement factor B; CFD, complement factor D.

vascular imaging techniques, such as 2-dimensional vascular ultrasound and coronary computed tomography angiography, as previously described (Fernández-Friera et al, 2015; Gonzalez-Cantero et al, 2022). Subclinical atherosclerosis was defined by the presence of 2 or more plaques in the different arterial territories assessed by vascular ultrasound imaging and coronary computed tomography angiography on the basis of similar approaches in the general population (Martínez-López et al, 2020; Núñez et al, 2022).

Coronary computed tomography angiography of the coronary arteries was performed with a 320-detector computerized tomography scanner (Aquilion ONE VISION, Toshiba) at HM Hospitales (Madrid, Spain), following the guidelines of the National Institutes of Health Radiation Exposure Committee. All coronary computed tomography angiography images were analyzed at the Advanced Cardiac Imaging Unit of HM Hospitales, and specific analyses were performed at The Laboratory of Inflammation and Cardiometabolic Diseases, National Heart, Lung and Blood Institute (Bethesda, MD) for quality control purposes.

Sample collection

At baseline, patients were weighed, were measured, and had their blood pressure taken. Fasting blood samples were collected, processed, and stored at −80 °C for subsequent high-throughput -omics analysis and biobanking. To ensure adequate tracking of all the procedures, each sample was assigned a unique identifier using a laboratory information management system (Bio-e-Bank), which was managed by the Ramón y Cajal Institute for Health Research (Instituto Ramón y Cajal de Investigación Sanitaria).

Proteomics analysis

The proteomics study was carried out on a sample of 18 patients with psoriasis, 9 of whom had atherosclerosis. The results were

validated in a different group of 44 patients, 23 of them with atherosclerosis. All patients were matched in terms of baseline characteristics (Tables 1 and 2).

Detailed protocols for quantitative proteomic analysis, including sample preparation, protein digestion and isobaric labeling, and protein identification and quantification are shown in Supplementary Materials and Methods.

Functional annotation clustering

To examine the function of the proteins identified, the list of 31 proteins of interest was analyzed with the online David Bioinformatics Resources software (2021 update, National Institutes of Health), assessing their function. Functional annotation clustering was performed to avoid any redundancy of enriched categories and pathways. Cord graph was plotted by https://www.bioinformatics.com.cn/en, a free online platform for data analysis and visualization, representing biological process terms according to gene ontology.

Western blotting

Plasma samples were obtained from a different cohort of patients with psoriasis with and without atherosclerosis. Equal amounts of plasma protein from each patient were resolved by 8−10% SDS-PAGE. After electrophoresis, the proteins were transferred to a nitrocellulose membrane under a constant voltage of 20 V, and the membranes were then stained with Ponceau S to guarantee that an equal amount of protein was loaded for each patient. The membranes were then blocked with 7.5% nonfat dry milk and probed overnight with the corresponding primary antibody. The primary antibodies used were rabbit polyclonal antisera raised against C3 (1/5000, Abcam, ab181147), C4BPB (1/5000, Abcam, ab199430), CFB (1/1000, ab133765), CFD (1/2000, Abcam, ab204917), and CFI (1/5000, Abcam, ab278524). After washing, the membranes were incubated with a specific horseradish peroxidase−conjugated secondary antibody, and antibody binding was detected by enhanced chemiluminescence (GE Healthcare), according to the manufacturer's instructions. Densitometry was performed with the ImageQuantTL software (GE Healthcare).

ELISA

Commercial ELISA kits were used to determine the plasma concentration of the HBA (Abcam, ab219049) and HBB (Abcam, ab235654), C6 (Abcam, ab230934), C8a (Abcam, ab288173), CFHR2 (Novus Biological, NBP2-75247), and DPP4 (Abcam, ab252365). The manufacturer's instructions were followed for each kit, and absorbance was measured at 450 nm. The concentration of each sample was determined from standard curves.

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences 15.0 software for Windows (SPSS). Continuous variables, such as age, were expressed as the mean ± SD. After demonstrating a normal distribution of the population using a Kolmogorov−Smirnov test, a comparison of the means was performed using a Student *t*-test. Discrete variables, such as sex or the presence/absence of risk factors, were expressed as percentages and compared between the groups using a Fisher's exact test. Statistical significance was accepted when $P < .05$. Receiver operating characteristic curves were generated using Statistical Package for the Social Sciences 15.0 for Windows software (SPSS).

DATA AVAILABILITY STATEMENT

Datasets related to this article can be found at https://data.mendeley.com/datasets/tk84fwj4cf/draft?a=985716c3-d915-4d95-bc98-8b053cd1f473, an opensource online data repository hosted at Mendeley Data.

ORCIDs

Laura Mourino-Alvarez: http://orcid.org/0000-0002-3928-1554
Inés Perales-Sanchez: http://orcid.org/0009-0009-9205-0956
Emilio Berna-Rico: http://orcid.org/0000-0003-0348-5421
Carlota Abbad-Jaime de Aragon: http://orcid.org/0000-0002-5587-1204
Nerea Corbacho-Alonso: http://orcid.org/0000-0002-5911-9589
Tamara Sastre Oliva: http://orcid.org/0000-0002-2399-2773
Cristina Juarez-Alia: http://orcid.org/0000-0001-7454-720X
Asunción Ballester-Martinez: http://orcid.org/0000-0002-6780-0390
Maria Castellanos-Gonzalez: http://orcid.org/0000-0001-6742-1019
Mar Llamas-Velasco: http://orcid.org/0000-0002-1187-1341
Pedro Jaen: http://orcid.org/0000-0002-7334-0044
Jorge Solis: http://orcid.org/0000-0002-4227-7062
Leticia Fernandez-Friera: http://orcid.org/0000-0002-4237-2166
Neha N. Mehta: http://orcid.org/0000-0003-4939-5130
Joel M. Gelfand: http://orcid.org/0000-0003-3480-2661
Maria G. Barderas: http://orcid.org/0000-0003-4290-4721
Alvaro Gonzalez-Cantero: http://orcid.org/0000-0001-8060-4784

CONFLICT OF INTEREST

AG-C has served as a consultant for AbbVie, Janssen, Novartis, Almirall, Celgene, and LEO Pharma, receiving grants/other payments, outside the submitted work. ML-V has served as an advisory board member, consultant, and research support as well as participated in clinical trials and honorary for speaking with the following pharmaceutical companies: Abbvie, Almirall, Amgen, Boehringer, Celgene, Janssen, Leo, Lilly, Kyowa kirin, Novartis, and UCB. AB has served as an advisory board member as well as participated in clinical trials and honorary for speaking with the following pharmaceutical companies: Abbvie, Almirall, Amgen, Boehringer, Celgene, Janssen, Leo, Lilly, Novartis, and Argnx. NNM has served as a consultant for Amgen, Eli Lilly, and Leo Pharma, receiving grants/other payments; as a principal investigator and/or investigator for AbbVie, Celgene, AstraZeneca, Janssen Pharmaceuticals, Novartis, and Abcentra, receiving grants and/or research funding; and as a principal investigator for the National Institutes of Health, receiving grants and/or research funding. JMG served as a consultant for Abbvie, BMS, Boehringer Ingelheim, Celldex (DSMB), FIDE (which is sponsored by multiple pharmaceutical companies), GSK, Happify, Lilly (DMC), Leo, Janssen Biologics, Neumentum, Novartis, Pfizer, UCB (DSMB), Neuroderm (DSMB), Regeneron, Trevi, Mindera Dx, and Veolia North America, receiving honoraria; receives research grants (to the Trustees of the University of Pennsylvania) from Amgen, Boehringer Ingelheim, and Pfizer; and received payment for continuing medical education work related to psoriasis that was supported indirectly by pharmaceutical sponsors. JMG is a copatent holder of resiquimod for treatment of cutaneous T-cell lymphoma. JMG is a Deputy Editor for the *Journal of Investigative Dermatology*, receiving honoraria from the Society for Investigative Dermatology; is Chief Medical Editor for Healio Psoriatic Disease (receiving honoraria); and is a member of the Board of Directors for the International Psoriasis Council, receiving no honoraria. The remaining authors state no conflict of interest.

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21/180501/000078). These results are aligned with the Spanish initiative on the Human Proteome Project.

AUTHOR CONTRIBUTIONS

Conceptualization: AG-C, MGB, EB-R, CA-J, JS, AB-M, PJ, LF-F, NNM, JMG; Data Curation: AG-C, MGB, EB-R, CA-J; Formal Analysis: LM-A, IP-S, NC-A, TSO, CJ-A, MGB, AG-C; Funding Acquisition: MGB, AG-C; Investigation: LM-A, IP-S, NC-A, TSO, CJ-A, MGB, AG-C, LF-F, EB-R, CA-J; Methodology: LM-A, IP-S, MGB, AG-C, AB-M, ML-V, MC-G, JS, LF-F, MGB, NNM; Project Administration: AG-C, MGB, CA-J, EB-R; Resources: AG-C, MGB, JS, LF-F, NMM; Software: AG-C, MGB, MT, NNM; Supervision: AG-C, MGB, JMG, NNM; Validation: LM-A, IP-S, NC-A, TSO, CJ-A, MGB, AG-C, MT, NNM; Visualization: LM-A, IP-S, NC-A, TSO, CJ-A, MGB, AG-C; Writing – Original Draft Preparation: LM-A, I-PS, MGB, AG-C; Writing - Review and Editing: AG-C, MGB, MC-G, ML-V, EB-R, CA-J, AB-M, JS, LF-F, MGB, PJ, JMG, NNM, LM-A, IP-S, NC-A, TSO, CJ-A

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2023.10.031.

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SUPPLEMENTARY MATERIALS AND METHODS

Quantitative proteomics analysis

Protein digestion and isobaric labeling. For the quantitative mass spectrometry (MS) analysis, 60 μg of protein of each depleted plasma was digested with trypsin using the Filter-Aided Sample Preparation (FASP) method (Wiśniewski et al., 2009). Tryptic peptides were evaporated and reconstituted in 100 μl 20 mM triethylammonium bicarbonate buffer (Sigma-Aldrich). Tandem mass tags (TMT) 6plex isobaric label reagents (Thermo Fisher Scientific) were reconstituted with 41 μl acetonitrile (ACN) (Thermo Fisher Scientific), and peptide labeling was carried out according to the manufacturer's instructions. Briefly, peptides were incubated with the labels for 1 hour at room temperature in 3 randomized batches, and each individual labeling reaction was quenched with 5% hydroxylamine for 15 minutes before the batches were mixed into a single TMT 6-plex tube. The 3 mixes were cleaned by solid-phase extraction (SPE) in C18 cartridges (Agilent Technologies). The eluted, clean, labeled peptide mixes were frozen and evaporated. Each 6-plex mix (corresponding to ∼360 μg initial protein) was reconstituted with 200 μl 5 mM ammonium formate (pH 10)/2% ACN before fractionation in a High-Performance Liquid Chromatography (HPLC)1100 UVVis (Agilent Technologies), with an XBridge Peptide BEH C18 column (130 Å, 5 μm, 2.1 × 100 mm: Waters). The following high-pH reversed phase (HP-RP) chromatographic gradient was established to separate the labeled peptides at a 200 μl/min flow rate for a total run time of 76 min, using solvent A (5 mM ammonium formate [pH 10]/2% ACN) and B (5 mM ammonium formate [pH 10]/90% ACN). Fractions were collected every 2 minutes (400 μl), and the fractions collected from 20−62 minutes were considered for further analysis. These fraction mixes were frozen and evaporated in a speedvac before liquid chromatography−tandem MS (LC-MS/MS) analysis.

For protein identification and quantification, an Orbitrap-Velos (Thermo Fisher Scientific) MS system equipped with a nanoESI ion source was used here. Each sample was loaded onto the chromatographic system, consisting of a C18 preconcentration cartridge (Agilent Technologies) connected to a 15 cm long, 100 μm i.d.;C18 column (Nikkyo Technos). Separation was achieved at 0.4 μl/min with a 120-minute ACN gradient from 3 to 35% (solvent A, 0.1% formic acid; solvent B, ACN/0.1% formic acid). The High-Performance Liquid Chromatography (HPLC) system was comprised of an Agilent 1200 capillary nano pump, a binary pump, a thermostated microinjector, and a micro switch valve. The Orbitrap-Velos was operated in the positive ion mode with a spray voltage of 1.8 kV. The MS analysis was performed in a data-dependent mode, acquiring an Orbitrap-full scan followed by a 10 tandem MS scan of the 10 most intense signals detected in the MS scan in higher-energy collisional dissociation (HCD) mode. The full MS was acquired in the Orbitrap with a resolution of 60,000 and a tandem MS at 7500.

The identification of the peptides was carried out in the Uniprot database, restricted to humans and contaminants, and using Proteome Discoverer, versión 1.4 software with a 1% false discovery rate. Protein quantification was carried out on the basis of the intensity of the reporter ions derived from TMT labeling, and only unique peptides (peptides that belong to 1 protein) were taken into account. The TMT intensity of the reporter ions was normalized to the total abundance of each TMT label to minimize the error due to the distinct protein loading of each channel. In the second step, central tendency normalization was also applied.

Sample preparation

To enhance the detection of less abundant proteins in the proteomics analysis, the abundant proteins were depleted from the plasma samples using High Select HSA/Immunoglobulin Depletion Mini Spin Columns (Thermo Fisher Scientific), according to the manufacturer's instructions. Briefly, 10 μl of plasma was incubated with the column resin for 10 minutes at room temperature. The depleted plasmas were then eluted by centrifugation, and protein abundance was quantified by the microBCA method (Thermo Fisher Scientific). Aliquots from each depleted plasma sample were kept at −40 °C until further use.

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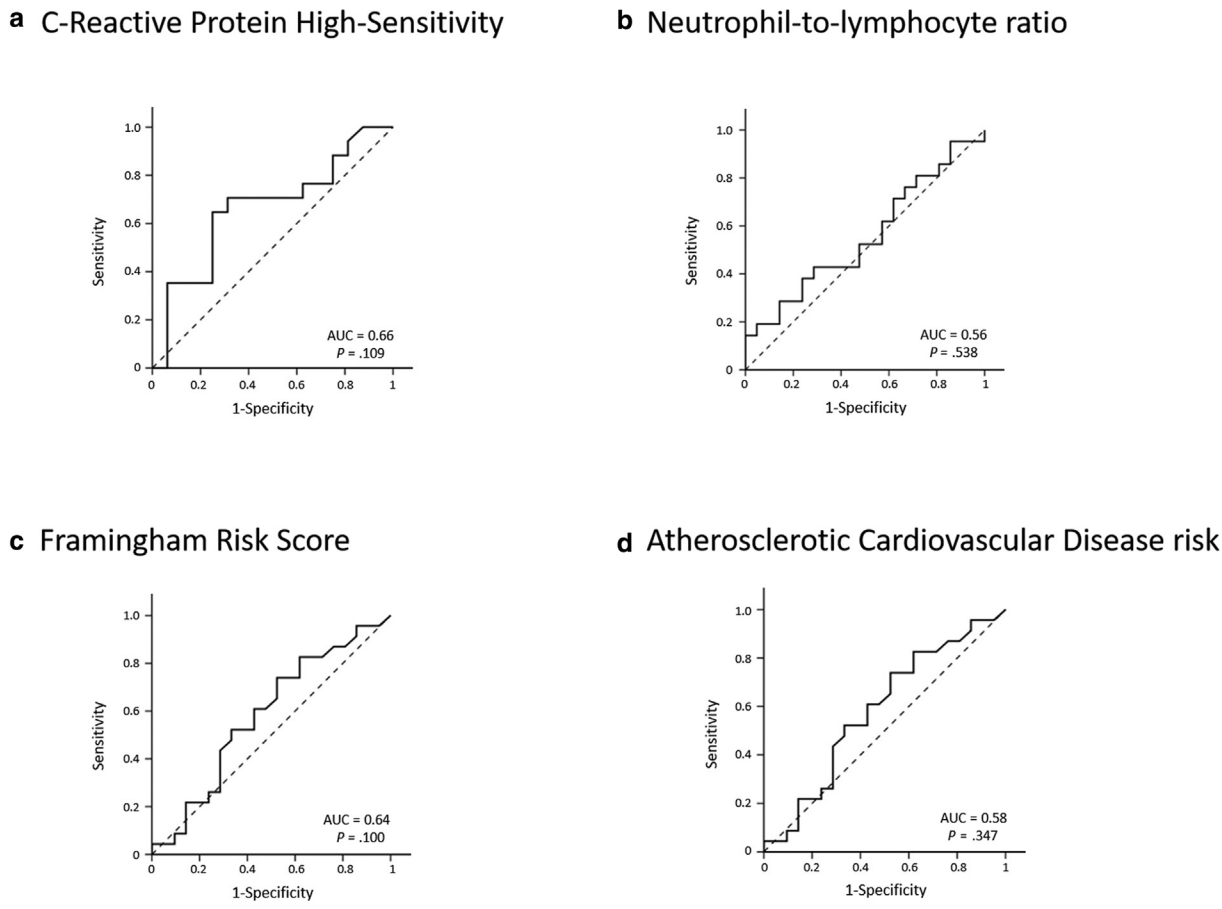

Supplementary Figure S1. ROC curves of different measurements and scores for cardiovascular risk assessment. C-reactive protein high-sensitivity (a), neutrophil-to-lymphocyte ratio (b), Framingham risk score (c), and atherosclerotic cardiovascular disease risk (d) have been assessed. The AUC and *P*-values are shown. AUC, area under the curve; ROC, receiver operating characteristic.