Investigating Systemic Inflammation and Hypercoagulability in Psoriasis: Implications for Cardiovascular Disease

by

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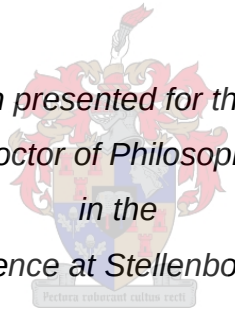

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This dissertation includes two original papers published in peer-reviewed journals and one unpublished publication. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

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Abstract

Psoriasis (PsO) is a common immune-mediated inflammatory disease of the skin, typically presenting as erythematous plaques covered with silvery scales. The condition is of multifactorial aetiology, encompassing interactions between environmental factors, genetic susceptibility, and dysregulated immune responses. The pathogenesis of PsO is predominantly driven by interleukin (IL)-17. In addition, inflammatory mediators associated with T helper (T_H) 1, T_H17, and T_H22 subsets are also overexpressed in psoriatic skin. Moreover, these inflammatory molecules may also be detected in the systemic circulation of patients with PsO. The disease is not solely limited to cutaneous sites, as multiple comorbidities have been linked to the condition. Notably, patients with PsO have been reported to have a significantly increased risk of cardiovascular disease (CVD). A potential mechanism that might contribute to this association is the presence of a hypercoagulable state – driven by persistent systemic inflammation – in these individuals. A pro-inflammatory milieu may favour coagulation, while suppressing natural anticoagulant mechanisms. In addition, inflammation may also alter – albeit indirectly – the fibrin clot structure and, by extension, the properties of the fibrin network. Therefore, the aim of this study was to assess the haemostatic profile and investigate potential alterations in fibrin clot structure in patients with PsO, compared to healthy individuals.

Whole blood samples were collected from patients with PsO (n=20) and healthy control subjects (n=20). The concentrations of blood-based markers of inflammation and endothelial and platelet activation were determined using enzyme-linked immunosorbent assays. Coagulation status was assessed by thromboelastography. The fibrin network architecture was analysed by scanning electron microscopy. Fibrin secondary structure was assessed through the examination of formalin-fixed, paraffin-embedded plasma clot sections using fluorescence microscopy and Fourier transform infrared (FTIR) spectroscopy. In order to detect areas rich in β-sheet structures, Congo red staining was performed, and sections were examined with brightfield and fluorescence microscopy. To determine if there were quantitative differences in the distribution of specific secondary structural elements in fibrin clots, FTIR analysis was conducted.

Elevated levels of inflammatory molecules (C-reactive protein, serum amyloid A, soluble intercellular adhesion molecule-1, and soluble P-selectin) were associated with PsO, thereby confirming the presence of systemic inflammation in patients with PsO. Thromboelastographic analysis revealed an increased tendency towards clot formation, that was also associated with disease presence. Moreover, the ultrastructure of fibrin clots from patients with PsO was

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altered – these clots were denser and consisted of thicker fibrin fibres, as compared to control subjects. Regarding the secondary structure of fibrin, the presence of β-sheet-rich areas, as identified by Congo red fluorescence, was detected in fibrin clots from both groups. Accordingly, FTIR analysis also did not show any significant differences between the secondary structure composition of fibrin clots from patients with PsO and those of healthy control subjects.

Taken together, the results of this study indicate that a hypercoagulable state is present in patients with PsO. This hypercoagulability seems to be a result of persistent systemic inflammation, rather than alterations to the molecular structure of fibrin. The hypercoagulable state in PsO might have implications for the management of CVD risk for individuals living with the condition.

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Opsomming

Psoriase (PsO) is ’n algemene immuun-bemiddelde inflammatoriese velsiekte wat tipies voorkom as rooierige verhewe vlekke bedek met silwerkleurige skubbe. Die toestand is van multifaktoriale etiologie, wat interaksies tussen omgewingsfaktore, genetiese vatbaarheid en wangereguleerde immuunresponse insluit. Die patogenese van PsO word hoofsaaklik deur interleukin (IL)-17 aangedryf. Daarbenewens word inflammatoriese mediators wat met T-helper (T_H) 1, T_H17 en T_H22 subversamelings geassosieer word, ook ooruitgedruk in psoriatiese vel. Boonop kan hierdie inflammatoriese molekules ook in die sistemiese sirkulasie van pasiënte met PsO opgespoor word. Die siekte is nie net beperk tot kutane areas nie, aangesien veelvuldige komorbiditeite aan die toestand gekoppel is. Daar is veral gerapporteer dat pasiënte met PsO ’n aansienlik verhoogde risiko vir kardiovaskulêre siekte (KVS) het. ’n Moontlike meganisme wat tot hierdie assosiasie kan bydra, is die teenwoordigheid van ’n hiperstolbare toestand – aangedryf deur kroniese sistemiese inflammasie – in hierdie individue. ’n Pro-inflammatoriese milieu kan stolling bevoordeel, terwyl natuurlike antistollingsmeganismes onderdruk word. Daarbenewens kan inflammasie ook die fibrienklontstruktuur – alhoewel indirek – en, by uitbreiding, die eienskappe van die fibriennetwerk verander. Daarom was die doel van hierdie studie om die hemostatiese profiel te assesseer en potensiële veranderinge in fibrienklontstruktuur in pasiënte met PsO, in vergelyking met gesonde individue, te ondersoek.

Heelbloed monsters is versamel vanaf pasiënte met PsO (n=20) en gesonde individue (n=20). Die konsentrasies van bloedgebaseerde inflammasiemerkers en merkers van endoteel- en bloedplaatjieaktivering is bepaal deur gebruik te maak van ensiemgekoppelde immunosorbenttoetse. Stollingstatus is deur trombo-elastografie geanaliseer. Fibriennetwerkargitektuur is deur skandeerelektronmikroskopie geanaliseer. Die sekondêre struktuur van fibrien is ondersoek deur die analise van formalien-gefikseerde paraffien-ingebedde plasma klontsnitte deur gebruik te maak van fluoressensiemikroskopie en Fourier transform infrarooi (FTIR) spektroskopie. Om areas ryk aan β-plaat strukture op te spoor, is Kongorooi kleuring uitgevoer, en snitte is ondersoek met helderveld- en fluoressensiemikroskopie. Om te bepaal of daar kwantitatiewe verskille in die verspreiding van spesifieke sekondêre strukturele elemente in fibrienklonte was, is FTIR-analise uitgevoer.

Verhoogde vlakke van inflammatoriese molekules (C-reaktiewe proteïen, serumamiloïed A, oplosbare intersellulêre adhesiemolekule-1 en oplosbare P-selektien) is met PsO geassosieer en die teenwoordigheid van sistemiese inflammasie in pasiënte met PsO word sodoende bevestig. Trombo-elastografiese analise het ’n verhoogde neiging tot klontvorming aan die lig

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gebring, wat ook geassosieer is met siekte teenwoordigheid. Boonop was die ultrastruktuur van fibrienklonte van pasiënte met PsO veranderd – hierdie klonte was digter en het bestaan uit dikker fibrienvesels, in vergelyking met kontrolegroeppasiënte. Met betrekking tot die sekondêre struktuur van fibrien is die teenwoordigheid van β-plaatryke areas, soos geïdentifiseer deur Kongorooi fluoressensie, opgespoor in fibrienklonte van beide groepe. Gevolglik het FTIR-analise ook geen betekenisvolle verskille tussen die sekondêre struktuursamestelling van fibrienklonte van pasiënte met PsO en dié van gesonde kontrolepasiënte getoon nie.

In samevatting dui die resultate van hierdie studie aan dat ’n hiperstolbare toestand teenwoordig is in pasiënte met PsO. Hierdie hiperstolbaarheid wil voorkom om 'n resultaat te wees van konstante sistemiese inflammasie, eerder as gevolg van veranderinge aan die molekulêre struktuur van fibrien. Die hiperstolbare toestand in PsO kan implikasies hê vir die besturing van KVS risiko in individue wat lewe met die kondisie.

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List of Abbreviations

A	Alpha (Angle)
ACC/AHA	American College of Cardiology and the American Heart Association
ADAMTS13	a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13
AMP	antimicrobial peptide
APC	activated protein C
BSA	body surface area
$CaCl_2$	calcium chloride
CAM	cell adhesion molecule
CANTOS	Canakinumab Anti-inflammatory Thrombosis Outcome Study
CASPAR	ClAssification of criteria for Psoriatic ARthritis
CCL	C-C motif chemokine ligand
CCR	C-C motif chemokine receptor
CD	cluster of differentiation
CD40L	CD40 ligand
cDC	conventional dendritic cell
CI	confidence interval
COLCOT	Colchicine Cardiovascular Outcomes Trial
CRP	C-reactive protein
CVD	cardiovascular disease
CXCL	C-X-C motif chemokine ligand
DAPSA	Disease Activity in PSoriatic Arthritis
DMARD	disease-modifying anti-rheumatic drug
DNA	deoxyribonucleic acid
DVT	deep vein thrombosis
EULAR	European League Against Rheumatism
FTIR	Fourier transform infrared
gp130	glycoprotein 130
GWAS	genome-wide association study
HLA	human leukocyte antigen
HMDS	hexamethyldisilazane
HR	hazard ratio
HREC	Health Research Ethics Committee
HRP	horseradish peroxidase
ICAM-1	intercellular adhesion molecule-1
IFN	interferon
IL	interleukin
IL-17R	interleukin-17 receptor
IL-1R1	interleukin-1 receptor type 1
IL-6R	interleukin-6 receptor
JUPITER	Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin
K	kinetics
MA	maximum amplitude
MAPK	mitogen-activated protein kinase
MHC	major histocompatibility complex

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MI	myocardial infarction
MRTG	maximum rate of thrombus generation
NaCl	sodium chloride
NaOH	sodium hydroxide
NET	neutrophil extracellular trap
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
OR	odds ratio
OsO_4	osmium tetra-oxide
PAF	platelet activating factor
PAI-1	plasminogen activator inhibitor-1
PASI	Psoriasis Area and Severity Index
PBS	phosphate-buffered saline
pDC	plasmacytoid dendritic cell
PPP	platelet-poor plasma
PsA	psoriatic arthritis
PsO	psoriasis
PSORS	psoriasis susceptibility
R	reaction time
RNA	ribonucleic acid
S100	S100 calcium binding protein
SAA	serum amyloid A
SEM	scanning electron microscopy
sICAM-1	soluble intercellular adhesion molecule-1
sP-selectin	soluble P-selectin
sVCAM-1	soluble vascular cell adhesion molecule-1
TEG	thromboelastography
TF	tissue factor
TFPI	tissue factor pathway inhibitor
T_H	T helper
TLR	Toll-like receptor
TMB	tetramethylbenzidine
TMRTG	time to maximum rate of thrombus generation
TNF	tumour necrosis factor
TNFR	tumour necrosis factor receptor
tPA	tissue-type plasminogen activator
TTG	total thrombus generation
VCAM-1	vascular cell adhesion molecule-1
VTE	venous thromboembolism
WB	whole blood
WHO	World Health Organization

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Units of Measure

%	percent
deg	degree
°C	degree Celsius
cm^{-1}	wavenumber
dyn/cm^2	dyne per square centimetre
$dyn/cm^2/s$	dyne per square centimetre per second
g	relative centrifugal force
h	hour
IU/mL	international units per millilitre
kDa	kilodalton
kV	kilovolt
M	molar
min	minute
mL	millilitre
mm	millimetre
ng/mL	nanogram per millilitre
nm	nanometre
µg/mL	microgram per millilitre
µL	microlitre
µm	micrometre
$µm^2$	square micrometre

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Chapter 1: Introduction

I think it also necessary, in the present series of them, to express the scaly psora by distinctive appellation: for this purpose, the term psoriasis

(Willan, 1809)

Psoriasis (PsO) is a common chronic immune-mediated inflammatory skin disease characterised by well-demarcated, erythematous plaques covered with silvery-white scales. The impact of the condition extends beyond its physical symptoms, as it is also associated with a deterioration in patients' quality of life (Obradors et al., 2016). In 2014, member states of the World Health Organization (WHO) passed a resolution to recognise PsO as a serious non-communicable disease (WHO, 2014). As a result, the WHO Global Report on Psoriasis was released to emphasise the impact of the disease on public health (WHO, 2016). In addition, the report also identified areas that require further research – one of these being the association between PsO and cardiovascular disease (CVD).

Recently, it has become evident that the manifestations of PsO are not limited to cutaneous sites, as several systemic comorbidities have been associated with the condition (Takeshita et al., 2017; Srivastava et al., 2021). Notably, CVD has been shown to be more prevalent in patients with PsO, as compared to the general population (Gelfand et al., 2006; Gelfand et al., 2009; Ahlehoff et al., 2011). In addition, a dose-response relationship between CVD risk and PsO has been observed, as the risk of CVD increases with an increase in clinical severity. In recent guidelines by the American College of Cardiology and the American Heart Association (ACC/AHA), the presence of PsO was also identified as a risk-enhancing factor that should be considered in the estimation and management of CVD risk (Arnett et al., 2019). To date, the precise underlying pathogenic mechanisms that drive the relationship between PsO and CVD remain elusive. Elucidation of the specific mechanism(s) is of importance, as these may inform the management of patients with PsO and the development of novel therapies.

A potential link between PsO and CVD may be the presence of a hypercoagulable state, driven by chronic systemic inflammation, in individuals with PsO. It is well known that inflammation may activate the coagulation cascade, and vice versa. Findings from recent clinical trials, such as the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) (Ridker et al., 2017) and the Colchicine Cardiovascular Outcomes Trial (COLCOT) (Tardif et al., 2019), have also underscored the role of inflammation in the development of CVD. Furthermore, aside from initiating coagulation, inflammation may also alter the final product of the coagulation cascade, namely the fibrin clot. Altered fibrin clot structure and stability have been associated with a range of cardiovascular conditions (Undas and Ariëns, 2011; Bridge et al., 2014).

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The aim of this dissertation is to assess the haemostatic profile and to investigate potential alterations in fibrin clot structure in patients with PsO, compared to healthy individuals. The specific research objectives of this study are:

1. to determine the levels of markers of inflammation and endothelial and platelet activation in patients with PsO, compared to healthy individuals, using enzyme-linked immunosorbent assays;
2. to determine the coagulation status of patients with PsO by measuring whole blood viscoelastic properties of patients with PsO and healthy individuals, using thromboelastography;
3. to characterise the fibrin network architecture and fibrin fibre diameter in patients with PsO, compared to healthy individuals, through the use of scanning electron microscopy;
4. to determine if amyloid-like areas are present in fibrin clots (prepared from platelet-poor plasma) of patients with PsO, compared to clots from healthy individuals, using fluorescence microscopy; and
5. to quantify differences in the secondary structure of fibrin clots from patients with PsO, compared to clots from healthy individuals, through the use of Fourier transform infrared spectroscopy.

An overview of the most relevant published literature is presented in **Chapter 2**. The first part of this chapter focuses on the aetiology and pathophysiology of PsO, whereas the second part focuses on the increased prevalence of CVD in patients with PsO and the potential contribution of specific inflammatory molecules – implicated in psoriatic pathology – to this association. In **Chapter 3**, the levels of specific blood-based markers, coagulation status, and the fibrin network architecture of patients with PsO, compared to healthy control subjects, are assessed. In **Chapter 4**, potential alterations in fibrin secondary structure are investigated in clots from patients with PsO as compared to clots from healthy control subjects. In **Chapter 5**, findings are contextualised, and recommendations for further research are discussed. A summary diagram of the layout of this dissertation is provided in **Figure 1.1**.

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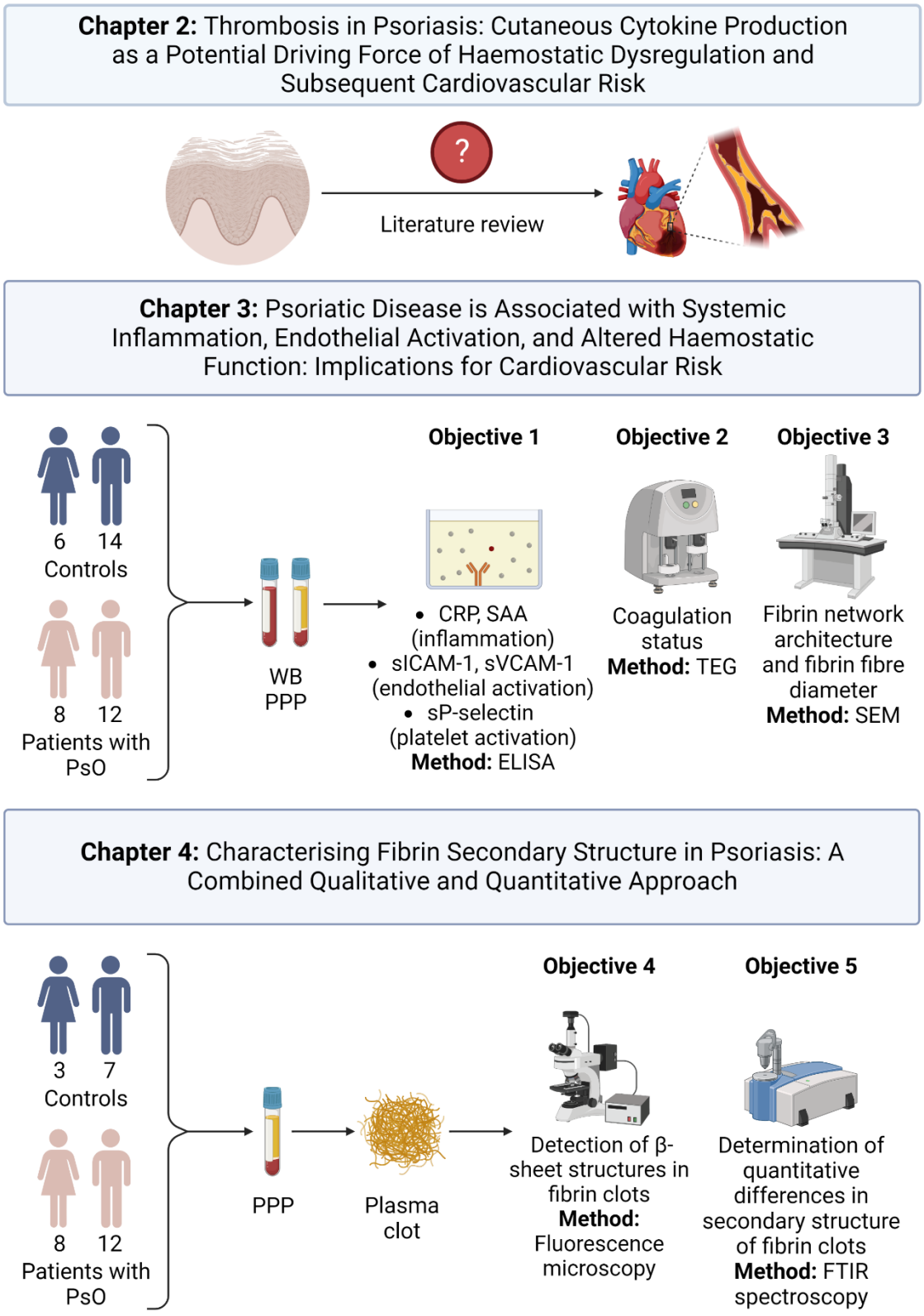

Figure 1.1 An overview of the structure of this dissertation. Diagram created with BioRender.com. **Abbreviations:** CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; FTIR, Fourier transform infrared; PPP, platelet-poor plasma; PsO, psoriasis; SAA, serum amyloid A; SEM, scanning electron microscopy; sICAM-1, soluble intercellular adhesion molecule-1; sP-selectin, soluble P-selectin; sVCAM-1, soluble vascular cell adhesion molecule-1; TEG, thromboelastography; WB, whole blood.

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Chapter 2: Thrombosis in Psoriasis: Cutaneous Cytokine Production as a Potential Driving Force of Haemostatic Dysregulation and Subsequent Cardiovascular Risk

A truncated version of this chapter has been published in Frontiers in Immunology (impact factor 8.786) under the title 'Thrombosis in Psoriasis: Cutaneous Cytokine Production as a Potential Driving Force of Haemostatic Dysregulation and Subsequent Cardiovascular Risk' (refer to **Appendix C: Publication**).
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Abstract

Psoriasis (PsO) is a common T cell-mediated inflammatory disorder of the skin with an estimated prevalence of 2%. The condition manifests most commonly as erythematous plaques covered with scales. The aetiology of PsO is multifactorial and disease initiation involves interactions between environmental factors, susceptibility genes, and innate and adaptive immune responses. The underlying pathology is mainly driven by interleukin (IL)-17. In addition, various inflammatory mediators from specific T helper (T_H) cell subsets, namely T_H1, T_H17, and T_H22, are overexpressed in cutaneous lesions and may also be detected in the peripheral blood of patients with PsO. Individuals living with PsO are at greater risk, compared to the general population, of developing multiple comorbid conditions. Cardiovascular disease (CVD) has been recognised as a prominent comorbidity of PsO. A potential mechanism contributing to this association may be the presence of a hypercoagulable state in individuals with PsO. Inflammation and coagulation are closely related. The presence of chronic, low-grade systemic inflammation may promote thrombosis – one of the major causes of CVD. A pro-inflammatory milieu may induce the expression of tissue factor, augment platelet activity, and perturb the vascular endothelium. Altogether, these changes result in a prothrombotic state. In this chapter, the aetiology and pathophysiology of PsO are described, as well as its relationship to CVD. Given the systemic inflammatory nature of PsO, the potential contribution of prominent inflammatory mediators – implicated in PsO pathogenesis – to establish a prothrombotic state is investigated.

2.1 Introduction

Psoriasis (PsO) is a chronic immune-mediated inflammatory disease of the skin, often associated with multiple comorbidities, affecting approximately 2% of the global population (Nestle et al., 2009). The reported prevalence of PsO in childhood may be up to 1.37% (Yang et al., 2007; Chen et al., 2008; Michalek et al., 2017), while the estimated prevalence in adults ranges from 0.51% to 11.43% (Danielsen et al., 2013; Takeshita et al., 2015; Michalek et al.,

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2017). Research suggests that disease development involves a complex interplay between genetic predisposition, environmental stimuli, and disordered innate and adaptive immune responses. Lesions can assume a variety of clinical forms (Naldi and Gambini, 2007), with plaque PsO being the most common disease variant. Typically, the condition manifests as well-circumscribed, erythematous papules and/or plaques covered with scales. In addition to its physical symptoms, PsO also imposes a significant psychosocial burden that may lead to anxiety, depression, and, in severe cases, suicidality (Kurd et al., 2010). The physical and psychological impact of PsO may significantly influence a patient's quality of life (Armstrong et al., 2012), as well as contribute to an increased risk of mortality in severe forms of the disease (Gelfand et al., 2007).

PsO is considered to be a T cell-mediated disease and the corresponding cytokine profile of psoriatic lesions indicates important roles of interferon (IFN)-α, interleukin (IL)-22, the IL-23/IL-17 axis, and tumour necrosis factor (TNF) in psoriatic pathology (Di Cesare et al., 2009; Hao, 2014; Grine et al., 2015). In addition to localised cutaneous inflammation, these molecules have also been detected in the systemic circulation of patients with PsO – increasing the risk of comorbidities (Reich, 2012; Oliveira et al., 2015a; Takeshita et al., 2017). Considering these observations, a paradigm shift has occurred from viewing PsO as merely 'skin-deep' to understanding it as a systemic inflammatory condition that can affect various extracutaneous tissues (Davidovici et al., 2010). Cardiovascular disease (CVD) is a notable comorbidity in patients with PsO. An increased risk of major cardiovascular events, as well as an increased CVD mortality in severe forms of the disease, has been reported in these individuals (Gelfand et al., 2006; Gelfand et al., 2009; Mehta et al., 2010; Ahlehoff et al., 2011; Manolis et al., 2019). The relationship between PsO and CVD is widely acknowledged; however, the mechanisms responsible remain uncertain.

Hypercoagulability is a potential mechanistic link accounting for the association between PsO and CVD. It is becoming increasingly clear that inflammation and coagulation are interrelated processes (Esmon, 2005; Foley and Conway, 2016). A hypercoagulable state may develop as a result of an imbalance in haemostatic and inflammatory activity mediated by pro-inflammatory cytokines. These molecules may promote the initiation of coagulation, inhibit endogenous anticoagulant systems, and impair fibrinolytic activity (Esmon, 2005; Levi and van der Poll, 2005). The vascular endothelium also plays a central role in clot formation, as it is located at the nexus of inflammation and coagulation. Under normal physiological conditions, the resting endothelium exhibits anticoagulant and anti-inflammatory effects (Abeyama et al., 2005; Iwaki et al., 2005). This homeostasis is disrupted in inflammatory conditions as a result of pro-inflammatory cytokines perturbing the vessel wall, resulting in a transition to an activated state that favours coagulation (Bevilacqua et al., 1986).

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In this chapter, the genetic and environmental risk factors associated with the development of PsO, as well as the pathogenesis of the condition, will be discussed briefly. In addition, the literature describing the systemic inflammatory nature of PsO and its subsequent relationship to CVD will also be considered. Finally, the potential contribution of prominent inflammatory mediators implicated in PsO, to promote a prothrombotic state, will also be appraised.

2.2 The Aetiology of Psoriasis

To date, the exact aetiology of PsO has not been fully elucidated. It is proposed that PsO pathogenesis is caused by the complex interplay between genetic susceptibility, environmental stimuli, and dysregulated immunity. PsO has primarily been viewed as a T cell-mediated inflammatory disease and its status as a 'true' autoimmune disorder remains disputed. Nonetheless, potential epidermal autoantigens have recently been identified, namely cathelicidin/LL37 (Lande et al., 2014), a disintegrin-like and metalloprotease domain containing thrombospondin type 1 motif-like 5 (Arakawa et al., 2015), and phospholipase A_2 Group IVD (Cheung et al., 2016). The most prominent genetic and environmental risk factors that have been implicated in the development of PsO will be discussed briefly in the following sections.

2.2.1 Genetic Risk Factors

The important role of a genetic component in the molecular pathogenesis of PsO has been supported by various family-based studies (Abele et al., 1963), including twin studies. An increased disease concordance rate has been reported in monozygotic twins, compared to dizygotic twins (Farber et al., 1974; Duffy et al., 1993; Grjibovski et al., 2007). Despite this disease concordance, the incidence never reaches 100% – suggesting a role for environmental factors, in addition to genetic susceptibility in PsO development. The current view is that the mode of inheritance for PsO is multifactorial. Classic genome-wide linkage analysis has identified at least nine different chromosomal regions, termed psoriasis susceptibility (*PSORS1–PSORS9*) loci, which have shown statistically significant associations with PsO (Duffin et al., 2008). Multiple studies have validated *PSORS1* as the most important genomic region in PsO predisposition and that it may account for up to 35% of disease heritability (Trembath et al., 1997). *PSORS1* is located on human chromosome 6p21.3 within the region of the major histocompatibility complex (MHC). *Human leukocyte antigen (HLA)-Cw*0602* has been accepted as the most likely *PSORS1* disease allele (Nair et al., 2006). The HLA-C gene encodes a class 1 MHC protein and participates in the priming of cluster of differentiation (CD) 8^+ T cell immune responses. More than 80 PsO susceptibility loci have been identified in genome-wide association studies (GWASs), conducted mainly on European and Asian populations (Hwang et al., 2017). In 2007, in the first GWAS for PsO, two genes (*IL12B* and *IL23R*) were identified that were associated with PsO risk (Cargill et al., 2007).

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Specific pathways implicated in PsO pathogenesis that have been identified through GWASs include IFN signalling, the IL-23 pathway, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) cascade, and the regulation of T cell responses (Nair et al., 2009; Strange et al., 2010; Sun et al., 2010; Tsoi et al., 2012; Tsoi et al., 2017).

2.2.2 Environmental Risk Factors

Environmental triggers play a critical role in the onset and development of PsO in genetically predisposed individuals through the interaction with genes and the induction of epigenetic modifications (Zeng et al., 2017). Various environmental exposures have been associated with the initiation and/or exacerbation of psoriatic lesions. Stressful life events are well known to contribute to the initiation and aggravation of PsO. A potential explanation for stress-induced PsO onset or flares may be dysregulation of the activity of the hypothalamic–pituitary–adrenal axis (Richards et al., 2005; Evers et al., 2010). Obesity is another significant risk factor for PsO and body mass index has been demonstrated to correlate with disease severity (Duarte et al., 2013). Potential mediators of this association may include adipokines, such as leptin and resistin, which possess pro-inflammatory actions (Johnston et al., 2008). Furthermore, epidemiological studies have suggested an association between smoking and the development of PsO, based on the increased incidence of PsO reported among current and former smokers compared to non-smokers (Naldi et al., 2005; Li et al., 2012; Armstrong et al., 2014). Smoking may induce PsO by augmenting the existing systemic oxidative stress, interacting with immune cells, and altering gene expression (Armstrong et al., 2011; Attwa and Swelam, 2011). Bacterial infections, specifically by *Staphylococcus aureus* and *Streptococcus pyogenes,* are recognised triggers of PsO. β-haemolytic streptococci have been isolated more frequently from throat swabs from patients with PsO, compared to controls (Gudjonsson et al., 2003), and enterotoxins from *S. aureus* have been linked to more severe PsO (Tomi et al., 2005). Dysbiosis of both the gut and skin microbiome has become a recurrent theme in individuals living with PsO (Visser et al., 2019). Gut dysbiosis, characterised by a decrease in microbes with anti-inflammatory and immunomodulatory properties (Mosca et al., 2016; Hiippala et al., 2018), seems to be prevalent in these individuals (Eppinga et al., 2016; Codoñer et al., 2018). An altered skin microbiome has also been associated with the condition (Gao et al., 2008; Alekseyenko et al., 2013).

2.3 The Pathophysiology of Psoriasis

2.3.1 Clinical Features

The diagnosis of PsO relies mainly on the visual presentation of the disease and no diagnostic blood tests or histopathological examinations exist. The presentation of PsO is heterogeneous and significant interindividual variability may be observed. Different clinical variants of the

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condition include chronic plaque PsO, erythrodermic PsO, guttate PsO, inverse PsO, and pustular PsO (**Table 2.1**) (Naldi and Gambini, 2007). Different subtypes may also exist simultaneously in the same individual at a given point in time. Of the disease variants, chronic plaque PsO is the most prevalent, affecting about 90% of individuals suffering from the condition (Griffiths and Barker, 2007; Boehncke and Schon, 2015). Typically, plaque PsO manifests as sharply demarcated, erythematous papulosquamous lesions covered with loosely adherent silvery-white lamellar scales. Lesions are often distributed symmetrically and sites of predilection include the scalp, elbows, knees, and the lumbosacral region. The disease is not solely confined to cutaneous sites, as joint and nail involvement also occur frequently in these individuals. Approximately 30% of patients with PsO may subsequently develop an inflammatory arthritis, due to an underlying enthesitis (Mease et al., 2013), while half of patients with PsO may also suffer from nail disease (Armesto et al., 2011).

Table 2.1 Clinical variants of psoriasis and its characteristic manifestations.

Clinical variant	Manifestations
Chronic plaque PsO	Erythematous plaques covered with scales Predilection sites include scalp, elbows, knees, and lumbosacral region
Erythrodermic PsO	Erythema that involves at least 75% of body surface area Potentially life-threatening
Guttate PsO	Lesions appear as small droplets Mainly involves trunk and limbs Preceded by upper respiratory tract infection
Inverse PsO	Fissured plaques, typically without scales Mainly involves flexural and intertriginous sites
Pustular PsO	Presents as sterile pustules Two forms: generalised pustular PsO and localised pustular PsO

Abbreviations: PsO, psoriasis.

2.3.2 Histopathological Features

The histopathological hallmarks of lesional skin in patients with PsO include acanthosis, hyperkeratosis, parakeratosis, elongation of the rete ridges, and the presence of a diverse inflammatory infiltrate in the dermal and epidermal compartments (Schön and Boehncke, 2005; Griffiths and Barker, 2007; Lowes et al., 2007; Raychaudhuri et al., 2014; Boehncke and Schon, 2015). The epidermis becomes markedly thickened (acanthosis), due to the hyperproliferation of keratinocytes (hyperkeratosis). The rapid maturation of keratinocytes results in aberrant terminal differentiation (parakeratosis) of these cells. Consequently, the abnormal retention of nuclei by keratinocytes can be observed in the stratum corneum. Rete

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ridges also become elongated, extending into the underlying dermis. Collections of neutrophils, namely Munro's micro-abscesses and the spongiform pustules of Kogoj, may also be observed in the stratum corneum and epidermis, respectively (Steffen, 2002). Remodelling of the cutaneous microvasculature, due to the expression of pro-angiogenic factors (Bhushan et al., 1999), is another characteristic feature of PsO (Simonetti et al., 2006).

2.3.3 Immunopathogenesis

Environmental factors (e.g., stress, obesity, smoking, infection, and dysbiosis) or physical trauma (the Koebner phenomenon) may perturb keratinocytes, resulting in the release of self-deoxyribonucleic acid (DNA) or self-ribonucleic acid (RNA) (Lande et al., 2007; Ganguly et al., 2009). In turn, these self-nucleic acids may form a complex with the endogenous antimicrobial peptide (AMP), LL37 (Lande et al., 2007). DNA-LL37 complexes activate plasmacytoid dendritic cells (pDCs), via Toll-like receptor (TLR) 9 signalling, which secrete high levels of IFN-α (Nestle et al., 2005). In a similar fashion, RNA-LL37 complexes activate pDCs in a TLR7-dependent manner (Ganguly et al., 2009). Consequently, conventional dendritic cells (cDCs), which function as professional antigen-presenting cells, are activated by IFN-α. RNA-LL37 complexes also possess the capacity to activate cDCs directly, leading to the production of IL-6 and TNF by these cells. Once activated, cDCs migrate to secondary lymphoid organs and secrete IL-12 and IL-23 (Oppmann et al., 2000), which induce the differentiation of naïve $CD4^+$ T cells into T helper (T_H) 1 (Nestle et al., 1994) and T_H17 (Zaba et al., 2009) cells, respectively. The expansion of T_H17 cells and IL-17 production are also mediated by IL-1β, which is produced by stressed keratinocytes and cDCs (Cai et al., 2014; Cai et al., 2019). This process provides a potential mechanism for the initiation of the inflammatory sequelae in PsO and highlights the central role of DCs to establish a link between the innate and adaptive branches of the immune system.

T_H1 cells express cytokines such as IFN-γ, IL-2, and TNF (Nestle et al., 1994; Austin et al., 1999). IFN-γ induces the expression of several chemokines and cytokines in the skin and also promotes the accumulation and infiltration of inflammatory cells (Nograles et al., 2008; Johnson-Huang et al., 2012). TNF has been identified as a key regulatory molecule in the cytokine network of PsO. In addition to T_H1 cells, a distinct subset of DCs, namely TNF- and inducible nitric oxide synthase-producing DCs, also release large amounts of TNF (Lowes et al., 2005). It has been suggested that TNF production mediates the proliferation of resident T cells in the development of psoriatic lesions (Boyman et al., 2004). In addition, TNF regulates the expression of cell adhesion molecules (CAMs), which mediate the extravasation of leukocytes, on endothelial cells and keratinocytes in psoriatic skin (Terajima et al., 1998). TNF also stimulates keratinocytes to induce the expression of pro-inflammatory cytokines such as IL-6, IL-8, and TNF itself, through the activation of NF-κB (Young et al., 2008). Furthermore,

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TNF may synergise with IL-17 to enhance the expression of key inflammatory genes in keratinocytes (Chiricozzi et al., 2011).

T_H17 cytokines such as IL-17A, IL-17F, and IL-22 (Liang et al., 2006; Zheng et al., 2007) are key pathogenic effectors in PsO. Therefore, PsO has been regarded as a T_H17-mediated disease. However, this paradigm is shifting towards understanding PsO as an IL-17-driven disease (Brembilla et al., 2018). Various other cellular sources of IL-17 exist, including $CD8^+$ T cells (comprising mucosa-associated invariant T cells and conventional T cells) (Teunissen et al., 2014), dermal γδ T cells (Cai et al., 2011), group 3 innate lymphoid cells (Villanova et al., 2014), mast cells, and neutrophils (Lin et al., 2011). IL-17A has been shown to govern the expression of signature PsO genes in keratinocytes (Muromoto et al., 2016). IL-17 acts on keratinocytes to induce the expression of AMPs, such as β-defensin 2, and neutrophil chemoattractants, namely C-X-C motif chemokine ligand (CXCL) 1, CXCL3, CXCL5, CXCL6, and CXCL8 (Nograles et al., 2008). Specifically, IL-17F promotes neutrophil accumulation in the dermis by stimulating the release of IL-8 by keratinocytes (Watanabe et al., 2009). T_H17 cytokines (IL-17A, IL-22, and TNF) also cause keratinocytes to produce C-C motif chemokine ligand (CCL) 20 and its receptor, C-C motif chemokine receptor (CCR) 6, thereby facilitating the recruitment and infiltration of T_H17 cells (Harper et al., 2009). IL-17A-induced keratinocyte production of IL-19 has been shown to upregulate the production of antimicrobial proteins, namely S100 calcium-binding protein (S100) A7, S100A8, and S100A9. IL-19 and IL-17A may also interact synergistically to enhance the keratinocyte response. These observations seem to suggest IL-19 as a potentially novel component of the IL-23/IL-17 axis (Witte et al., 2014), which plays a crucial role in the development of psoriatic inflammation (van der Fits et al., 2009). Apart from its role in T_H17 differentiation and expansion, IL-23 has been demonstrated to induce dermal inflammation and epidermal hyperplasia – mediated through the combined effects of IL-17A (Rizzo et al., 2011) and IL-22 (Zheng et al., 2007).

IL-22 is co-expressed with IL-17A and IL-17F by activated T_H17 cells (Liang et al., 2006; Zheng et al., 2007) as well as T_H22 cells (Eyerich et al., 2009). IL-22 does not affect immune cells (Wolk et al., 2004) and primarily targets epithelial cells, mediating innate immune responses and contributing to wound healing (Zenewicz and Flavell, 2011). This cytokine has a dual nature, exhibiting both anti- and pro-inflammatory properties (Alabbas et al., 2018). IL-22 induces the overexpression of AMPs, such as β-defensin 2, and the S100 protein family (Boniface et al., 2005; Liang et al., 2006; Tohyama et al., 2018). In addition, IL-22 has been reported to downregulate the expression of genes involved in the regulation of keratinocyte differentiation, resulting in acanthosis (Boniface et al., 2005; Wolk et al., 2006; Wolk et al., 2009). As a pro-inflammatory cytokine, IL-22 stimulates keratinocyte production of chemokines, namely CCL2, CCL20, CXCL5, and CXCL8 (Boniface et al., 2005; Tohyama et al., 2018). These chemotactic agents will promote the infiltration of monocytes and/or

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macrophages (Vestergaard et al., 2004), neutrophils (Gillitzer et al., 1996), and T cells (Homey et al., 2000) at sites of cutaneous inflammation. A graphical representation of key cytokine circuits in the immunopathogenesis of PsO is provided in **Figure 2.1**.

Other cytokines that have been implicated in psoriatic inflammation include IL-9, IL-19, IL-20, IL-24, IL-33, and IL-36. IL-9 has been suggested to contribute to PsO pathogenesis via its pro-angiogenic activity and induction of IL-17 production (Singh et al., 2013). In addition, members of the IL-20 subfamily of cytokines (IL-19, IL-20, and IL-24) have been shown to affect keratinocyte proliferation and differentiation and to induce the expression of various PsO-related molecules (Sa et al., 2007). Indeed, suppression of these cytokines resulted in alleviation of epidermal hyperplasia in patients with PsO (Wang et al., 2012). Furthermore, IL-33 was recently demonstrated to act on keratinocytes in an autocrine manner, thereby perpetuating the psoriatic inflammatory response (Zeng et al., 2021). Finally, IL-36 has been implicated in keratinocyte-specific pathways that mediate dermal inflammation in PsO (Goldstein et al., 2020; Hernández-Santana et al., 2020).

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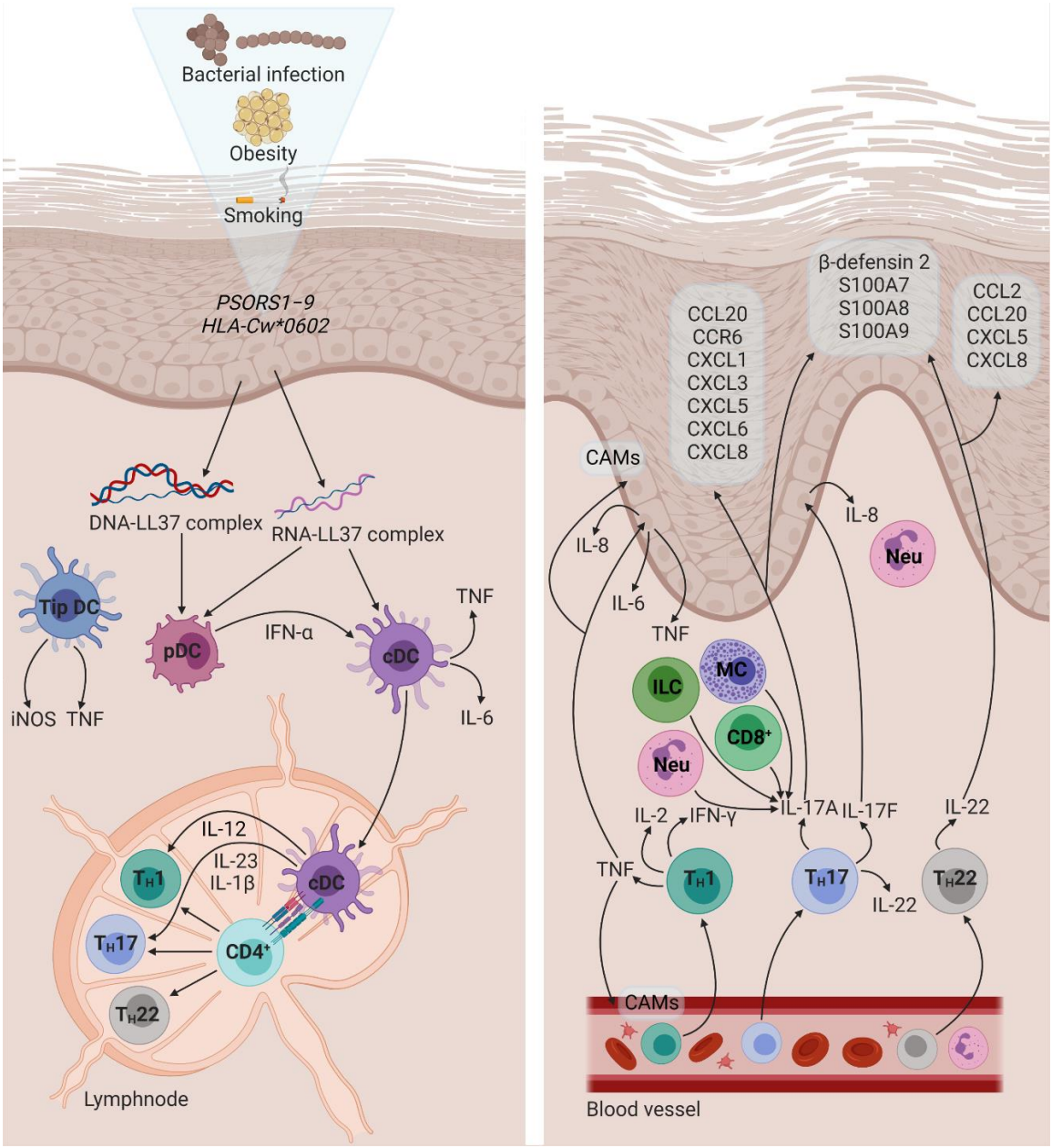

Figure 2.1 The immunopathogenesis of psoriasis. A combination of genetic and environmental factors activates plasmacytoid dendritic cells. In turn, conventional dendritic cells activate naïve cluster of differentiation (CD) 4^+ T cells through the presentation of an unknown antigen. Subsequently, activated $CD4^+$ T cells differentiate into T helper (T_H) 1, T_H17, and T_H22 cells, which migrate to the dermis and give rise to a psoriatic plaque. Diagram created with BioRender.com. **Abbreviations:** CAM, cell adhesion molecule; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; CD, cluster of differentiation; cDC, conventional dendritic cell; CXCL, C-X-C motif chemokine ligand; DNA, deoxyribonucleic acid; *HLA*, human leukocyte antigen; IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; iNOS, inducible nitric oxide synthase; MC, mast cell; Neu, neutrophil; pDC, plasmacytoid dendritic cell; *PSORS*, psoriasis susceptibility; RNA, ribonucleic acid; S100, S100 calcium-binding protein; T_H, T helper; Tip DC, tumour necrosis factor- and inducible nitric oxide synthase-producing dendritic cell; TNF, tumour necrosis factor.

2.4 Treatment of Psoriasis

When determining the appropriate treatment regimen for a patient with PsO, various factors should be taken into consideration, such as disease severity, the presence of psoriatic arthritis, comorbidities, and the impact on the patient's quality of life. The severity of PsO may be

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determined by the percentage of the total body surface area (BSA) that is involved, with <3% BSA considered mild, 3–10% considered moderate, and >10% considered severe. Mild to moderate PsO is treated with topical agents including corticosteroids, calcineurin inhibitors, vitamin D_3 analogues, keratolytic agents, anthralin, retinoids, and coal tar preparations (Elmets et al., 2021). For moderate to severe PsO, phototherapy and systemic treatments are prescribed (Armstrong and Read, 2020). Oral systemic therapies, such as methotrexate, apremilast, cyclosporine, and acitretin, possess anti-inflammatory and immunomodulatory properties (Menter et al., 2020). The advent of biologic systemic therapies has drastically changed the treatment of PsO. Currently, there are four classes of biologicals available, namely TNF inhibitors, IL-12/23 inhibitors, IL-17 inhibitors, and IL-23 inhibitors (Griffiths et al., 2021). These agents exert their effects by targeting prominent cytokines involved in the pathogenesis of PsO. When compared to traditional systemic agents, these therapies have better safety profiles and may also be more efficacious (Kaushik and Lebwohl, 2019).

2.5 The Systemic Inflammatory Nature of Psoriasis

PsO was initially primarily regarded as a hyperkeratotic disorder solely confined to affected skin areas. However, the systemic inflammatory nature of the condition has become increasingly apparent in recent years (Grozdev et al., 2014; Korman, 2020). Various pro-inflammatory molecules are overexpressed in psoriatic skin lesions. These mediators also seem to be released into the systemic circulation of patients with PsO and may reflect disease severity (Arican et al., 2005). Peripheral inflammation is evident by the presence of a host of inflammatory molecules in the blood of these individuals (Arican et al., 2005; Rashmi et al., 2009; Bai et al., 2018; Cataldi et al., 2019). Moreover, inflammation may also be detected at extracutaneous sites. In a pilot study by Mehta et al. (2011), ^{18}F-fluorodeoxyglucose positron emission tomography-computed tomography was utilised to localise and quantify inflammatory activity in individuals with moderate to severe PsO. The authors detected systemic inflammation in the skin, joints, liver, and vasculature, with significantly greater aortic and hepatic inflammation in patients with PsO compared to age- and gender-matched control subjects (Mehta et al., 2011). Furthermore, individuals with PsO have an increased risk of developing multiple comorbid diseases (presented in **Table 2.2**), which serves as further evidence for the presence of systemic inflammation in the condition. It has been proposed that the chronic course of PsO, as well as common inflammatory molecules and/or overlapping pathways, may act as the driving forces of the development and/or worsening of these extracutaneous manifestations (Reich, 2012; Grozdev et al., 2014).

Besides systemic comorbidities, individuals with PsO may also develop metabolic abnormalities. An increased prevalence of metabolic syndrome – a constellation of dyslipidaemia, hypertension, insulin resistance, and visceral obesity – has been reported in

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patients with PsO compared to healthy controls (Sommer et al., 2006; Cohen et al., 2007; Cohen et al., 2008b; Tobin et al., 2010; Langan et al., 2012). However, the directionality of these associations remains unknown. It is possible that PsO may initiate inflammatory pathways that drive the development of metabolic disturbances. Inflamed adipose tissue produces adipocytokines (Fantuzzi, 2005) that may worsen existing PsO (Gerdes et al., 2011). It should be noted that metabolic dysfunction may also contribute to an increased cardiovascular burden in individuals with PsO. This is, however, beyond the scope of this review.

Table 2.2 Comorbidities associated with psoriasis.

Comorbidity	Selected references
Chronic kidney disease	Wan et al., 2013; Chi et al., 2015; Chiu et al., 2015
Cardiovascular disease	Gelfand et al., 2006; Gelfand et al., 2009; Ahlehoff et al., 2011
Depression	Kurd et al., 2010; Dowlatshahi et al., 2014
Diabetes	Cohen et al., 2008a; Armstrong et al., 2013a; Khalid et al., 2013
Inflammatory bowel disease	Cohen et al., 2009; Li et al., 2013; Egeberg et al., 2016a
Malignancy	Brauchli et al., 2009; Pouplard et al., 2013; Egeberg et al., 2016b
Metabolic syndrome	Sommer et al., 2006; Love et al., 2011; Langan et al., 2012
Non-alcoholic fatty liver disease	Gisondi et al., 2009; Candia et al., 2015; Roberts et al., 2015

2.6 The Association Between Psoriasis and Cardiovascular Disease

In recent years, CVD has emerged as a particularly prominent comorbidity of PsO. Several epidemiological studies have reported an increased risk of CVD in individuals living with PsO, compared to the general population. Furthermore, recent guidelines for the primary prevention of CVD, by the American College of Cardiology and the American Heart Association (ACC/AHA) indicated PsO as a risk-enhancing factor for the development of atherosclerotic CVD (Arnett et al., 2019).

In a landmark study by Gelfand and colleagues (2006), patients with PsO were reported to have a higher incidence of myocardial infarction (MI). Patients with mild and severe PsO had an incidence of 4.04 [95% confidence interval (CI): 3.88–4.21] and 5.13 (95% CI: 4.22–6.17) per 1000 person-years, compared to an incidence of 3.58 in healthy control subjects (95% CI: 3.52–3.65) (Gelfand et al., 2006). In addition, traditional CVD risk factors such as hyperlipidaemia, hypertension, and smoking were more prevalent among patients with PsO. PsO remained an independent risk factor for CVD, after adjusting for established CVD risk factors. Similar associations were noted in studies assessing the risk of stroke (Gelfand et al., 2009) and venous thromboembolism (VTE) (Ahlehoff et al., 2011) in patients with PsO. Gelfand et al. (2009) reported an increased risk of stroke in mild [hazard ratio (HR) 1.06; 95% CI: 1.0–

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1.1] and severe (HR 1.43; 95% CI: 1.1–1.9) PsO, after adjusting for major risk factors. Furthermore, Ahlehoff and colleagues (2011) reported higher incidence rates of VTE in individuals living with PsO (1.92 and 3.20 per 1000 person-years for mild and severe PsO) compared to healthy controls (1.29 per 1000 person-years). In a meta-analysis by Gaeta and colleagues (2013), it was shown that PsO confers an excess risk of 24% for the development of CVD. It has also been reported that severe disease activity contributes substantially to increased mortality due to CVD (Mallbris et al., 2004; Abuabara et al., 2010; Armstrong et al., 2013b). Mehta et al. (2010) identified severe PsO as an independent risk factor for CVD deaths (HR 1.57; 95% CI: 1.26–1.96), after adjusting for traditional CVD risk factors. Moreover, in a study by Abuabara and colleagues (2010), it was determined that CVD was responsible for the highest absolute (61.9 deaths per 1000 person-years) and excess (3.1 deaths per 1000 person-years) risk in patients with PsO.

The link between PsO and CVD may potentially be explained by the chronicity of the disease and the associated systemic inflammation. A considerable body of literature supports the notion that chronic, low-grade systemic inflammation plays a central role in the pathogenesis and propagation of CVD (Kofler et al., 2005; Demetz and Ott, 2012; Nagareddy and Smyth, 2013; Golia et al., 2014). Moreover, elevated levels of C-reactive protein (CRP) – a sensitive marker of systemic inflammation – have been suggested as a predictor of future cardiovascular events (Marcovina et al., 2007). Two landmark trials, namely the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) (Ridker et al., 2008) and the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) (Ridker et al., 2017), also provided evidence for a prominent role of systemic inflammation in the etiopathogenesis of CVD. In the following section, the interplay between inflammation and coagulation as well as how an imbalance in these activities may promote the development of CVD – specifically via thrombosis – will be elaborated upon.

2.7 Psoriatic Inflammation and Coagulopathy

Inflammation and coagulation are interdependent processes, demonstrated by the dynamic crosstalk between these systems. Under normal physiological conditions, these systems function as protective mechanisms and are tightly regulated. However, dysregulation may result in chronic, systemic inflammation and/or thrombotic complications. In response to invading pathogens or tissue damage, inflammation ensues to eliminate the original insult and to promote wound healing and tissue repair. However, if the inflammatory process is not duly resolved, acute inflammation may transition to chronic, systemic inflammation. As a result, the haemostatic balance may shift – driven primarily by pro-inflammatory cytokines – towards a procoagulant state.

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A particularly prominent molecule in the context of inflammation-induced coagulation is tissue factor (TF) (Levi and van der Poll, 2005). The tenase complex, comprising TF and factor VIIa, activates the extrinsic coagulation pathway, culminating in the generation of thrombin. In the final steps of coagulation, thrombin catalyses the conversion of soluble fibrinogen to insoluble fibrin. Pro-inflammatory cytokines may upregulate the expression of TF on endothelial cells and monocytes (Szotowski et al., 2005). In addition, inflammation favours the suppression of natural anticoagulant mechanisms, namely the antithrombin pathway, the protein C pathway, and tissue factor pathway inhibitor (TFPI) (Esmon, 2005; Levi and van der Poll, 2005). Finally, fibrinolytic activity may also decrease due to a continuous increase in plasminogen activator inhibitor-1 (PAI-1) levels stimulated by pro-inflammatory molecules (Levi and van der Poll, 2005).

In turn, coagulation may also modulate and perpetuate the inflammatory response. Coagulation proteases may bind to protease-activated receptors on the activated endothelium, inducing the synthesis and expression of CAMs (Camerer et al., 2000; Camerer et al., 2002). These molecules play a pivotal role in the extravasation of leukocytes to sites of inflammation. Furthermore, activated coagulation factors may also elicit an inflammatory response by interacting with immune cells to induce the production of cytokines (de Jonge et al., 2003; Vorlova et al., 2017). Platelets are also increasingly recognised for their ability to mediate and regulate inflammation. Activated platelets release their granular content, which comprises a plethora of procoagulant and pro-inflammatory molecules. Platelets are also implicated in the recruitment of leukocytes and the regulation of vascular permeability (Gros et al., 2014).

From these observations, it is evident that inflammation and coagulation should not be viewed as separate entities and that an imbalance in these activities may culminate in the development of prothrombotic state. Arterial and venous thrombotic events are the most frequent cause of CVD (Blann and Lip, 2006; Lozano et al., 2012; Raskob et al., 2014). Arterial thrombosis, which ensues after the rupture of an atherosclerotic plaque or damage to the vessel wall, may give rise to MI or stroke. Platelets play a central role in the formation of an arterial thrombus (Koupenova et al., 2017). In contrast, the pathomechanism of venous thrombosis, associated with deep vein thrombosis (DVT) and pulmonary embolism, is less clear. Virchow's triad describes three factors, namely hypercoagulability, endothelial dysfunction, and altered blood flow, which may predispose an individual to the development of venous thrombosis (Mackman, 2012).

As discussed in the previous sections, PsO is characterised by chronic, systemic inflammation and accompanied by an increased risk of CVD. A potential mechanism may be the development of a hypercoagulable state, via the action(s) of multiple pro-inflammatory cytokines, due to psoriatic pathology. The most prominent cytokines that could play a role in

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the development of such a prothrombotic state – IL-1β, IL-6, IL-17, and TNF – will be discussed below. The effects of these cytokines, specifically on platelets and the endothelium, will be considered. In **Figure 2.2** and **Figure 2.3** schematic representations are provided of the signalling pathways that could be initiated in platelets and endothelial cells, respectively, upon stimulation with these cytokines.

2.7.1 Interleukin-1β

IL-1 is a pro-inflammatory cytokine that participates in various biological activities, such as the innate immune response (Sims and Smith, 2010) and inflammation (Mantovani et al., 2019). Two IL-1 isoforms exist, namely IL-1α and IL-1β, and intracellular signalling is mediated through the same receptor, IL-1 receptor type 1 (IL-1R1) (Garlanda et al., 2013). IL-1 has been implicated in cutaneous inflammation (Murphy et al., 2000) and the IL-1β/IL-1R1 signalling pathway has been shown to play a fundamental role in psoriatic pathology by contributing to the differentiation of T_H17 cells (Mee et al., 2006; Cai et al., 2019). Accordingly, IL-1β is overexpressed in psoriatic lesions, as compared to non-lesional skin (Tamilselvi et al., 2013) and skin from control subjects (Cai et al., 2019). Furthermore, it has been demonstrated that plasma levels of IL-1β correlate with the clinical severity of PsO pre- and post-treatment (Tamilselvi et al., 2013). This cytokine has emerged as a significant mediator of CVD, as demonstrated by the results of the CANTOS trial. In this trial, inhibition of IL-1β by canakinumab (a human monoclonal antibody) resulted in a reduction of recurrent atherothrombotic events in patients with previous MI and raised CRP levels (Ridker et al., 2017).

A functional IL-1R1 is expressed on the platelet surface (Brown et al., 2013; Beaulieu et al., 2014). Upon stimulation with IL-1β, platelets synthesise and release additional IL-1β, which may be shed in association with platelet microparticles (Lindemann et al., 2001; Brown et al., 2013). In addition, this cytokine also augments agonist-induced platelet aggregation via phosphorylation of the p38 mitogen-activated protein kinase (MAPK) pathway (Beaulieu et al., 2014). Platelet adhesion to substrates such as collagen and fibrinogen is also enhanced by IL-1β (Beaulieu et al., 2014).

IL-1β may activate the vascular endothelium to initiate a pro-inflammatory program and acquire procoagulant properties. Cytokines with procoagulant effects, such as IL-6 and IL-8, are expressed and secreted by endothelial cells (Kuldo et al., 2005; Máko et al., 2010). It is proposed that the expression of these molecules is mainly under the control of MAPK and NF-κB signalling. IL-1β also promotes TF activity (Archipoff et al., 1991; Herbert et al., 1992) and diminishes thrombomodulin activity (Archipoff et al., 1991) on endothelial cells. Thrombomodulin plays an important role in the protein C pathway. Under normal physiological conditions, protein C binds to the thrombin-thrombomodulin complex, which results in

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generating activated protein C (APC). In turn, the coagulation factors Va and VIIIa are inactivated by the action of APC (Esmon, 2003). Finally, IL-1β has been reported to suppress fibrinolysis by inhibiting the synthesis and secretion of tissue-type plasminogen activator (tPA), while upregulating PAI-1 secretion by the endothelium (Schleef et al., 1988; Larsson et al., 2008). The suppression of tPA transcription is mainly mediated by NF-κB signalling (Larsson et al., 2008). Both of these molecules play a role in regulating fibrinolytic activity. Therefore, dysregulation of the expression of these proteins may contribute to an increased susceptibility to thrombosis.

2.7.2 Interleukin-6

IL-6 is a pleiotropic cytokine involved in several physiological processes including the acute-phase response and antibody production (Tanaka et al., 2014). The effects of IL-6 are mediated via two receptors, namely the IL-6 receptor (IL-6R) and the signal-transducing subunit, glycoprotein 130 (gp130) (Tanaka et al., 2014). In PsO, IL-6, combined with IL-23, contributes to the differentiation of T_H17 cells (Di Cesare et al., 2009). The overexpression of this cytokine has been detected in psoriatic skin lesions (Goodman et al., 2009) as well as in the circulation of individuals living with PsO (Neuner et al., 1991; Arican et al., 2005; Oliveira et al., 2015b). In addition, IL-6 signalling has been suggested to diminish regulatory T cell activity in PsO, thereby allowing the expansion of effector T cells (Goodman et al., 2009). In the context of CVD, IL-6 has emerged as a pivotal mediator of thrombotic disease. Raised IL-6 levels have been found to be related to recurrent venous thrombosis (van Aken et al., 2000), with detectable levels of IL-6 associated with a two-fold increase in the risk of venous thrombotic events (Reitsma and Rosendaal, 2004). The potential involvement of IL-6 in the development of adverse cardiovascular events is further supported by findings of a sub-study of the CANTOS trial. In this study, baseline IL-6 levels were associated with an increased risk of cardiovascular events. More importantly, it was demonstrated for the first time that lowering IL-6 levels – via the inhibition of IL-1β – resulted in a 15% reduction of atherothrombotic events (MI and stroke) (Ridker et al., 2018).

During inflammation, IL-6 acts on hepatocytes to induce the synthesis of acute-phase proteins such as CRP, fibrinogen, and serum amyloid A (SAA) (Heinrich et al., 1990). Both CRP and SAA have demonstrated the ability to promote coagulation via the induction of TF synthesis in endothelial cells and suppression of the TFPI pathway (Zhao et al., 2007; Chen et al., 2009). With regard to fibrinogen, it has been suggested that a causal relationship exists – at least in murine models – between hyperfibrinogenaemia and thrombosis (Machlus et al., 2011). Elevated concentrations of fibrinogen may also contribute to an increase in clot stability and resistance to fibrinolysis (Machlus et al., 2011).

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Although platelets do not express IL-6R, they have been shown to express gp130 (Marta et al., 2005; Marino et al., 2013). Upon thrombin-induced platelet activation, the soluble form of IL-6R is secreted which can form a complex with IL-6 (Marta et al., 2005; Marino et al., 2013). This complex may activate gp130, in a process termed trans-signalling, resulting in the activation of signal transducer and activator of transcription 3 (Marino et al., 2013). However, IL-6 trans-signalling does not seem to affect platelet activation and/or aggregation. Nevertheless, IL-6 may promote platelet adhesion and aggregation indirectly. IL-6 inhibits the activity of a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13 (ADAMTS13) (Bernardo et al., 2004). In turn, ADAMTS13 is responsible for the cleavage of ultra-large von Willebrand factor multimers released by the endothelium. In the ultra-large form, these multimers are hyperreactive and may interact with platelets to induce adhesion and aggregation.

2.7.3 Interleukin-17

IL-17 plays a pivotal role in the innate immune response, particularly in host defence against microbial invasion (Jin and Dong, 2013). The IL-17 family consists of six members, namely IL-17A to IL-17F, and signalling is mediated through the IL-17 receptor (IL-17R) family that comprises five members, IL-17RA to IL-17RE (Kolls and Lindén, 2004). This pro-inflammatory cytokine has been implicated in the pathogenesis of diverse autoimmune and inflammatory diseases, including PsO (Onishi and Gaffen, 2010). Indeed, IL-17 is regarded as the principal driver of psoriatic inflammation. The overexpression of this cytokine has been detected in lesional psoriatic skin, when compared to non-lesional skin (Johansen et al., 2009; Kolbinger et al., 2017). In addition, serum levels of IL-17A are elevated in patients with PsO (Oliveira et al., 2015b) and may also reflect disease severity (Kolbinger et al., 2017). With respect to cardiovascular pathology, conflicting results have been reported on the role of IL-17. Some studies have found IL-17 to have a protective effect (Simon et al., 2013), while others have reported a pro-atherogenic role of IL-17 (Erbel et al., 2009). Nevertheless, the prothrombotic effects of IL-17 have been demonstrated. In a murine model of DVT, IL-17A was found to promote thrombus formation by enhancing platelet aggregation and neutrophil infiltration of thrombi (Ding et al., 2018). Moreover, it has been shown that targeted blocking of IL-12/23 and IL-17 resulted in improved skin phenotype and lengthened clotting times to occlusive thrombus formation in a murine model of psoriatic disease (Li et al., 2018).

Platelets have been demonstrated to express a functional IL-17 receptor, namely IL-17RA (Maione et al., 2011). Exposing activated platelets (induced by adenosine diphosphate) to IL-17 augments platelet aggregation (Maione et al., 2011; Hot et al., 2012; Zhang et al., 2012). This action is suggested to be mediated by the opening of the mitochondrial permeability transition pore (Yuan et al., 2015) and the phosphorylation of extracellular signal-regulated

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kinase-2 (Zhang et al., 2012; Yuan et al., 2015). Furthermore, stimulation with IL-17 causes an increased expression and accelerated externalisation of P-selectin and exposure of the $\alpha_{IIb}\beta_3$ integrin (Maione et al., 2011; Zhang et al., 2012; Yuan et al., 2015). Both of the aforementioned molecules facilitate platelet aggregation and are used as markers of platelet activation.

IL-17 may also alter the endothelium towards an activated state. IL-17RA is constitutively expressed in endothelial cells (Zhu et al., 2011). It has been reported that IL-17 and TNF act synergistically to activate the endothelium, resulting in the synthesis of inflammatory mediators, and the expression of CAMs [E-selectin and intercellular adhesion molecule-1 (ICAM-1)] and TF by these cells (Hot et al., 2012). Anticoagulant activity by the endothelium also becomes diminished, as thrombomodulin expression is downregulated (Hot et al., 2012). It should be noted that IL-17 alone is not a strong inducer of inflammatory activity, however, IL-17, in concert with TNF, potently induces pro-inflammatory gene expression. This effect is thought to be mediated by the ability of IL-17 to stabilise messenger RNA (Hartupee et al., 2007; Sun et al., 2011). Finally, the vascular expression of CD39, an inhibitor of platelet aggregation, is also downregulated by IL-17A (Hot et al., 2012; Maione et al., 2014).

2.7.4 Tumour Necrosis Factor

TNF is a potent inflammatory cytokine, orchestrating various processes such as inflammation, cell differentiation, and apoptosis (Zelová and Hošek, 2013). The effects of TNF are mediated by two receptors, namely TNF receptor (TNFR) 1 and TNFR2 (Baud and Karin, 2001). In PsO, this cytokine amplifies the inflammatory response via the generation of reactive oxygen species and inducing the expression of cytokines and CAMs (Terajima et al., 1998; Bonifati and Ameglio, 1999). Increased TNF activity has been detected in involved skin from patients with PsO (Ettehadi et al., 1994) and TNF levels were also elevated in the serum of these individuals (Arican et al., 2005; Kyriakou et al., 2014). In relation to cardiovascular conditions, increased concentrations of TNF have been associated with recurrent coronary events (Ridker et al., 2000) and venous thrombosis (Reitsma and Rosendaal, 2004). In addition, TNF levels have been suggested to be an independent predictor of cardiovascular events such as MI and stroke, and CVD mortality (Kablak-Ziembicka et al., 2011). Regarding the prothrombotic properties of TNF, discrepant results have been reported. In a murine model of atherothrombosis, potent antithrombotic effects of TNF were noted (Cambien et al., 2003). In contrast, another murine study found that treatment with this cytokine resulted in accelerated thrombus formation (Pircher et al., 2012).

It has been suggested that TNF may promote the (hyper)activation of platelets by interacting with TNFR1 and TNFR2, which is expressed on the platelet membrane (Pignatelli et al., 2005). TNF may also facilitate platelet activation by stimulating the arachidonic acid pathway

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(Pignatelli et al., 2005). Furthermore, CD40 ligand (CD40L) expression by activated platelets may be induced by TNF via an arachidonic acid-dependent oxidative stress mechanism (Pignatelli et al., 2004; Pignatelli et al., 2008). In turn, CD40L enhances platelet activation and aggregation, as well as thrombus formation (Yacoub et al., 2010). TNF also induces the release of large von Willebrand factor multimers from endothelial cells, which propagates platelet thrombus formation (Bernardo et al., 2004). Nevertheless, the ability of TNF to stimulate platelet activation directly remains contested, with some studies reporting no effect (Pircher et al., 2012).

TNF may alter the properties of the endothelium by inducing the synthesis of procoagulant molecules and suppressing natural anticoagulant mechanisms. Engagement of TNF with TNFR1 results in the expression of TF on the surface of endothelial cells and the subsequent deposition of fibrin (Nawroth and Stern, 1986; Conway et al., 1989; Kirchhofer et al., 1994; Pircher et al., 2012). In addition, the production of platelet activating factor (PAF), by the endothelium, is also induced by TNF (Camussi et al., 1987; Bussolino et al., 1988). PAF induces platelet aggregation (Chignard et al., 1979) and functions as a potent inflammatory mediator (Camussi et al., 1990). The activity of APC is dependent on the presence of functional endothelial thrombomodulin, which has been shown to be downregulated by TNF (Nawroth and Stern, 1986). Mechanistically, this inhibitory effect is mediated by downregulating the transcription of the thrombomodulin gene (Conway and Rosenberg, 1988; Pircher et al., 2012). Finally, TNF inhibits fibrinolysis by induction of the PAI-1 gene (Pircher et al., 2012) through the activity of NF-κB (Hou et al., 2004) and by decreasing the release of tPA (Schleef et al., 1988).

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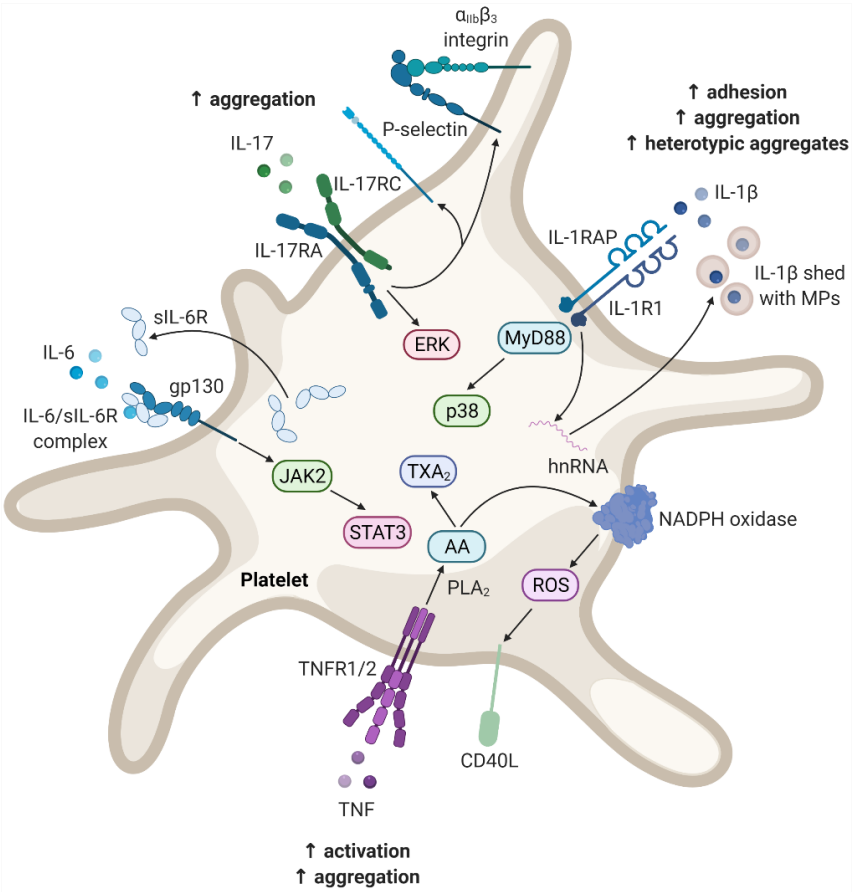

Figure 2.2 Potential signalling pathways involved in platelets, upon stimulation with interleukin (IL)-1β, IL-6, IL-17, and tumour necrosis factor. These pro-inflammatory mediators may contribute to platelet activation as well as enhancing the platelet response to agonist-induced platelet activation. Diagram created by BioRender.com. **Abbreviations:** AA, arachidonic acid; CD40L, cluster of differentiation 40 ligand; ERK, extracellular signal-regulated kinase; gp, glycoprotein; hnRNA, heterogeneous nuclear ribonucleic acid; IL, interleukin; IL-17RA, interleukin-17 receptor A; IL-17RC, interleukin-17 receptor C; IL-1R1, interleukin-1 receptor type 1; IL-1RAP, interleukin-1 receptor accessory protein; JAK2, Janus kinase 2; MP, microparticle; MyD88, myeloid differentiation primary response 88; NADPH, nicotinamide adenine dinucleotide phosphate; PLA_2, phospholipase A_2; ROS, reactive oxygen species; sIL-6R, soluble interleukin-6 receptor; STAT3, signal transducer and activator of transcription 3; TNF, tumour necrosis factor; TNFR1/2, tumour necrosis factor receptor 1/2; TXA_2, thromboxane A_2.

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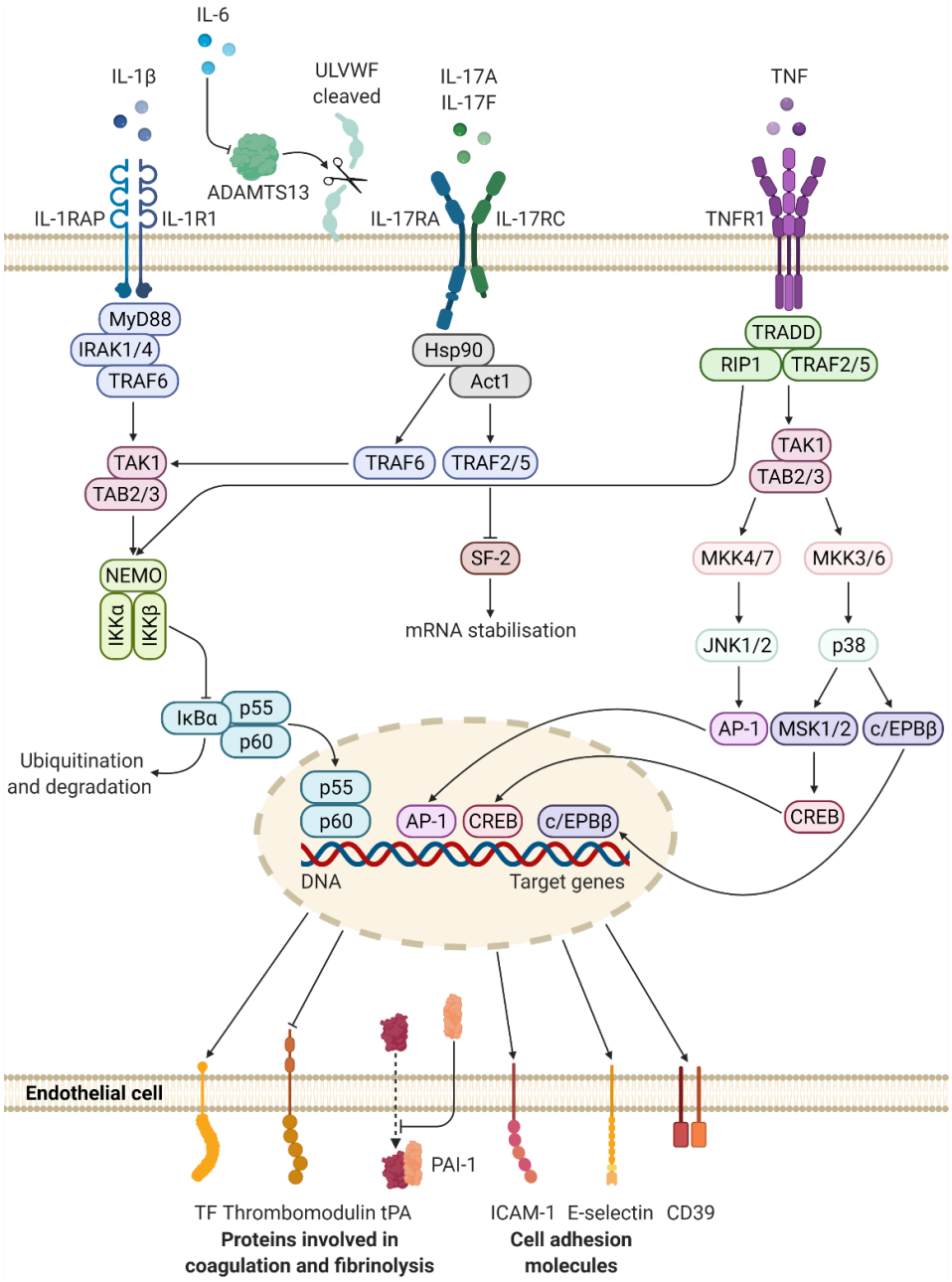

Figure 2.3 Potential intracellular signalling pathways involved in endothelial cells, upon stimulation with interleukin (IL)-1β, IL-6, IL-17, and tumour necrosis factor. These mediators may upregulate the synthesis and release of molecules that promote platelet adhesion and coagulation, while suppressing the expression of proteins involved in fibrinolysis. Altogether these changes lead to a prothrombotic state. Diagram created by BioRender.com. **Abbreviations:** ADAMTS13, a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13; AP-1, activator protein-1; c/EPBβ, CCAAT/enhancer-binding protein β; CD39, cluster of differentiation 39; CREB, cyclic adenosine monophosphate response element binding protein; DNA, deoxyribonucleic acid; Hsp90, heat shock protein 90; ICAM-1, intercellular adhesion molecule-1; IKBα, NF-kappa-B inhibitor alpha; IKK, inhibitor of nuclear factor kappa-B kinase; IL, interleukin; IL-17RA, interleukin-17 receptor A; IL-17RC, interleukin-17 receptor C; IL-1R1, interleukin-1 receptor type 1; IL-1RAP, interleukin-1 receptor accessory protein; IRAK1/4, interleukin-1 receptor-associated kinase 1/4; JNK1/2, c-Jun N-terminal kinase 1/2; MKK, mitogen-activated protein kinase kinase; MSK1/2, mitogen- and stress-activated protein kinase 1/2; MyD88, myeloid differentiation primary response 88; NEMO, NF-kappa-B essential modulator; PAI-1, plasminogen activator inhibitor-1; RIP1, receptor-interacting protein kinase 1; SF-2, splicing factor-2; TAB2/3, TAK1-binding protein 2/3; TAK1, transforming growth factor-β-activated kinase 1; TF, tissue factor; TNF, tumour necrosis factor; TNFR1, tumour necrosis factor receptor 1; tPA, tissue-type plasminogen activator; TRADD, tumour necrosis factor receptor 1-associated death domain protein; TRAF, tumour necrosis factor receptor-associated factor; ULVWF, ultra-large von Willebrand factor.

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2.8 A Possible Role for Fibrin(ogen) and Altered Clot Structure

As described in the preceding sections, dysregulated inflammatory molecules in PsO may act in concert to disrupt the carefully maintained haemostatic balance by favouring the initiation of coagulation and the suppression of fibrinolysis. The focal point of this pathophysiological state is the final common pathway of the coagulation cascade, in which the soluble glycoprotein fibrinogen is enzymatically converted to insoluble fibrin monomers by the serine protease thrombin. Polymerisation of fibrin monomers along with covalent cross-linking, mediated by factor XIIIa, results in the formation of a gel-like meshwork. The fibrin network, together with the primary platelet plug, constitute a haemostatic plug or blood clot. Despite being vital to preserve vascular integrity, fibrin formation may become perturbed under inflammatory conditions, resulting in an increased risk of thrombosis (Zabczyk et al., 2017) – a major determinant of CVD risk. To date, the fibrin network in PsO has not been fully characterised.

Determinants of a prothrombotic fibrin clot phenotype have however been shown to be present in PsO. Elevated fibrinogen levels may contribute to the pathogenesis of thrombosis by promoting the formation of a denser clot (Machlus et al., 2011). Such increased concentrations of fibrinogen have been detected in patients with PsO (Vanizor Kural et al., 2003; Rocha-Pereira et al., 2004; Arias-Santiago et al., 2012). This is a result of the acute-phase response, which is primarily mediated by IL-6 (Heinrich et al., 1990). The fibrinogen concentration also determines the mechanical properties of a fibrin clot, with raised levels resulting in enhanced structural integrity (Machlus et al., 2011). Another factor influencing the fibrin architecture is the thrombin concentration. A high concentration of thrombin promotes the formation of a less-porous clot, consisting of densely packed thin fibrin fibres (Wolberg et al., 2003). This type of network conformation is also more resistant to fibrinolysis (Collet et al., 2000). Importantly, the pro-inflammatory molecules considered in this review have all been shown to induce the generation of cellular TF, thereby potentially contributing to an increase in thrombin generation. It has also been reported that pro-inflammatory mediators, such as CRP (Salonen et al., 1984) and SAA (Page et al., 2019), may interact with fibrin(ogen), potentially altering fibrin clot structure and properties.

2.9 Concluding Remarks

PsO is an immune-mediated inflammatory disorder of the skin, characterised by the overexpression of T_H1-, T_H17-, and T_H22-derived inflammatory cytokines. These mediators are upregulated in the lesional skin of patients with PsO and may also be released into the circulatory system of these individuals. The systemic inflammatory nature of the condition is reflected by a multitude of dysregulated inflammatory molecules, which may give rise to various comorbidities. PsO has been identified as an independent risk factor for the development of CVD (**Figure 4.2**). The chronic subclinical systemic inflammation associated with the condition

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may predispose individuals living with PsO to thrombosis – the leading cause of CVD. Inflammatory cytokines may contribute to the development of a prothrombotic state, via the induction of TF, platelet activation and/or enhancing the platelet response, and endothelial dysfunction. The exact mechanisms underlying the association between PsO and CVD remain elusive. However, it is imperative that both physicians and patients must be aware of the potential cardiovascular risk that PsO may pose. Therefore, effective management of the condition should not only aim to ameliorate cutaneous inflammation but also systemic inflammation, in order to prevent the development of secondary comorbidities such as CVD.

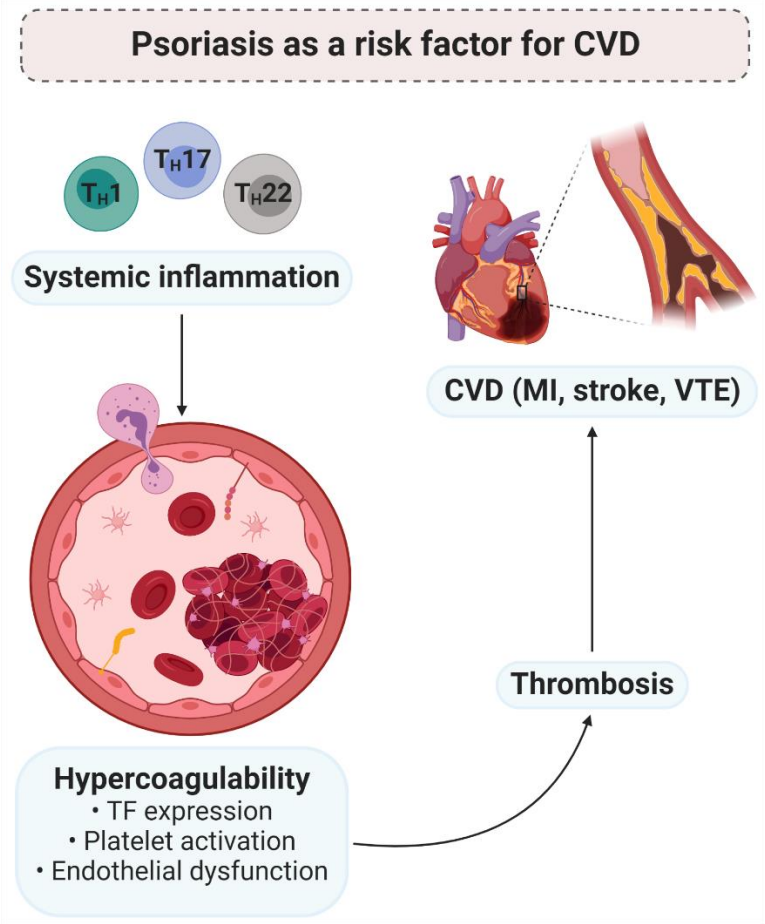

Figure 2.4 Psoriasis as a risk factor for cardiovascular disease (CVD). Chronic low-grade systemic inflammation, due to psoriatic pathology, may contribute to the development of a hypercoagulable state. Pro-inflammatory mediators induce the expression of tissue factor, enhance platelet activation and/or the platelet response, and endothelial dysfunction. In turn, hypercoagulability predisposes to thrombosis, which is the most frequent underlying pathology of CVD. Diagram created with BioRender.com. **Abbreviations:** CVD, cardiovascular disease; MI, myocardial infarction; TF, tissue factor; T_H, T helper; VTE, venous thromboembolism.

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Chapter 3: Psoriatic Disease is Associated with Systemic Inflammation, Endothelial Activation, and Altered Haemostatic Function: Implications for Cardiovascular Risk

A truncated version of this chapter has been published in Scientific Reports (impact factor 4.997) under the title 'Psoriatic disease is associated with systemic inflammation, endothelial activation, and altered haemostatic function' (refer to **Appendix D: Publication**). This article is licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits the use, adaptation, and distribution of the material. For a copy of this licence, please visit http://creativecommons.org/licenses/by/4.0/.

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Abstract

Psoriasis (PsO) is a chronic, immune-mediated inflammatory skin disease, associated with a significant cardiovascular burden. Hypercoagulability is a potential underlying mechanism that may contribute to the increased risk of major cardiovascular events in patients with PsO. Whole blood samples were collected from 20 patients with psoriatic disease and 20 healthy individuals. The concentrations of inflammatory molecules [C-reactive protein (CRP), serum amyloid A (SAA), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1, and soluble P-selectin (sP-selectin)] were determined by enzyme-linked immunosorbent assays. In addition, coagulation status was evaluated by thromboelastography (TEG). The fibrin network architecture was assessed by scanning electron microscopy (SEM). Elevated levels of circulating inflammatory molecules (CRP, SAA, sICAM-1, and sP-selectin) were significantly associated with the presence of PsO. Furthermore, an increased tendency towards clot formation (indicated by TEG parameters) also correlated significantly with disease presence. SEM revealed that fibrin clots from patients with psoriasis were denser, compared to clots from healthy controls, with an increased fibrin fibre diameter associated with PsO. These results add to the accumulating evidence of the systemic nature of PsO and the subsequent risk of cardiovascular comorbidities, potentially due to an acquired hypercoagulability.

3.1 Introduction

Psoriasis (PsO) is a chronic, inflammatory skin disease, with a worldwide prevalence of approximately 2% (Nestle et al., 2009). The condition commonly presents as erythematous, well-demarcated plaques covered by slivery-white scales. Of those affected, 30% may also develop an inflammatory arthritis [psoriatic arthritis (PsA)] (Mease et al., 2013). The cytokine network in PsO is primarily polarised towards the overexpression of T helper (T_H) 1 and T_H17 cytokines (Arican et al., 2005). Prominent inflammatory mediators implicated in the initiation

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and maintenance of the disease include interferon (IFN)-α, interleukin (IL)-22, the IL-23/IL-17 axis, and tumour necrosis factor (TNF) (Nestle et al., 2005; van der Fits et al., 2009; Wolk et al., 2009; Chiricozzi et al., 2011). Chronic systemic inflammation is evidenced by elevated levels of these and other inflammatory molecules in the blood of patients with PsO. Individuals living with PsO are also at an increased risk, when compared to the general population, of developing comorbidities such as depression, diabetes, inflammatory bowel disease, malignancy, metabolic syndrome, and cardiovascular disease (CVD) (Cohen et al., 2008a; Kurd et al., 2010; Ahlehoff et al., 2011; Langan et al., 2012; Pouplard et al., 2013; Egeberg et al., 2016a). It has been proposed that shared inflammatory pathways may act as a driving force for both PsO and its extracutaneous manifestations (Davidovici et al., 2010). Considering these observations, a paradigm shift has occurred from viewing PsO as merely 'skin-deep' to appreciating it as a systemic inflammatory condition (Arican et al., 2005; Dowlatshahi et al., 2013). A new concept, namely 'psoriatic disease', has been introduced to describe skin and joint manifestations as well as the involvement of various other organ systems in the same individual (Scarpa et al., 2006). Recently, the term 'psoriatic syndrome' has also been proposed to rather define the condition as a syndrome that comprises diverse clinical features that may or may not occur at different stages of the disease (Lubrano et al., 2019).

In recent years, CVD has been recognised as a prominent comorbidity of the condition. Various epidemiological studies have reported a significantly increased risk of major cardiovascular events, such as myocardial infarction (MI), stroke, and venous thromboembolism (VTE), in patients with PsO (Gelfand et al., 2006; Gelfand et al., 2009; Ahlehoff et al., 2011). Moreover, the presence of PsO has been identified as an independent risk factor for the development of CVD after adjusting for traditional risk factors (Gelfand et al., 2006; Gelfand et al., 2009; Mehta et al., 2010). Similarly, PsA also confers an increased risk for the development of cardiovascular events, such as MI and stroke (Ogdie et al., 2015). It has been shown that the 10-year risk of adverse cardiac events, as determined by the Framingham Risk Score, is generally underestimated in these individuals (Ernste et al., 2015). Moreover, it has been recommended by the European League Against Rheumatism (EULAR) task force that CVD risk should be evaluated regularly in patients with PsA, while also aiming to manage disease activity to lower the associated risk (Agca et al., 2017).

Hypercoagulability might be a potential mechanism underlying the relationship between PsO and CVD. The processes of inflammation and coagulation are interconnected, with these systems interacting in a bidirectional manner. A hallmark of sustained, low-grade, systemic inflammation is a shift in the haemostatic balance towards a prothrombotic state (Kell and Pretorius, 2015). Elevated levels of C-reactive protein (CRP), an acute-phase reactant and marker of inflammation, have been shown to be predictive of an increased risk of developing thrombotic disease (Tohgi et al., 2000; Danenberg et al., 2003; Folsom et al., 2009). Pro-

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inflammatory cytokines are the major mediators of inflammation-induced coagulation activation. These molecules disturb the haemostatic balance by inducing the expression of tissue factor (TF) on monocytes and endothelial cells, downregulating endogenous anticoagulant mechanisms, and impairing fibrinolytic activity (Levi et al., 2004; Margetic, 2012). TF plays a central role in coagulation, as it functions as a cofactor for factor VIIa in the extrinsic tenase complex. This complex initiates the extrinsic pathway of coagulation, resulting in the generation of thrombin. In the terminal stages of coagulation, soluble fibrinogen is converted into insoluble fibrin through the enzymatic action of thrombin. Fibrin plays a fundamental role in haemostasis, providing the structural scaffolding for blood clots. The fibrin network architecture is an important determinant of clot stability and fibrinolytic susceptibility (Lord, 2011). Compact, less permeable clots, consisting of thin fibrin fibres, are associated with an increased risk of thrombotic events. This prothrombotic fibrin clot phenotype has been associated with various thromboembolic diseases, such as coronary artery disease (Collet et al., 2006), stroke (Rooth et al., 2011), and VTE (Undas et al., 2009). Therefore, characterisation of the fibrin network architecture might be a useful biomarker for thrombosis.

The vascular endothelium serves as an important interface between inflammation and coagulation, playing an essential role in the regulation of these entities. Under physiological conditions, the intact endothelium exhibits anti-inflammatory properties and expresses anticoagulant proteins (Abeyama et al., 2005; Iwaki et al., 2005). However, upon stimulation with pro-inflammatory molecules, endothelial cells become activated (Bevilacqua et al., 1986). Consequently, the endothelium upregulates the expression of cell adhesion molecules (CAMs) [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1)], selectins (E-selectin and P-selectin), inflammatory mediators, and procoagulant factors, while attenuating the expression of anticoagulants. This functional deterioration of the endothelial barrier could exert a net prothrombotic effect. In addition, endothelial activation could promote the firm adhesion and full activation of platelets via the interactions between CAMs and/or selectins and platelet surface receptors (von Hundelshausen and Weber, 2007; Wagner and Frenette, 2008). The interaction of P-selectin with its receptor, P-selectin glycoprotein ligand-1, is a key mediator of endothelial–platelet interactions (Frenette et al., 1995) and the formation of platelet-leukocyte aggregates (von Hundelshausen and Weber, 2007). Endothelial dysfunction and platelet hyperactivity have been implicated in the pathogenesis of both arterial and venous thrombosis (Koupenova et al., 2017; Poredos and Jezovnik, 2017).

In this chapter, the haemostatic profile of patients with PsO, compared to healthy individuals, was investigated. To this end, whole blood (WB) coagulation efficiency was evaluated, and the fibrin network architecture was characterised. In addition, the levels of biomarkers indicative of inflammation, endothelial dysfunction, and platelet activation were measured.

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3.2 Materials and Methods

3.2.1 Ethical Clearance and Informed Consent

Ethical clearance for this study was received from the Health Research Ethics Committee (HREC) of Stellenbosch University, Stellenbosch, South Africa (HREC reference N19/03/043, refer to **Appendix A: Ethical Approval Letter**). Prior to WB collection, a written form of informed consent was obtained from all study participants (refer to **Appendix B: Participant Information Leaflet and Consent Form**). A unique number was assigned to every study participant to ensure confidentiality throughout the study. All methods were carried out in accordance with the guidelines of the relevant ethics committees. We strictly adhered to the Declaration of Helsinki.

3.2.2 Study Design and Study Population

This was a pilot exploratory study. A sample size from 10 to 40 participants per group has been recommended for pilot studies (Julious, 2005; Hertzog, 2008). A cross-sectional study design was followed. The study population consisted of n=40 volunteers, which included n=20 patients with psoriatic disease and n=20 healthy individuals. Patients with PsA were recruited during their visits to the Winelands Rheumatology Centre, Stellenbosch, for routine consultations. Of these patients, 18 had active psoriatic skin disease and 20 had active psoriatic arthritis. All patients were assessed by the same rheumatologist. PsO skin severity was assessed using the Psoriasis Area and Severity Index (PASI). For PsA, disease severity was assessed using the Disease Activity in PSoriatic Arthritis (DAPSA). According to DAPSA scores, PsA disease activity was classified as low, moderate, or high. Enthesitis was graded using the Leeds Enthesitis Index, while joint involvement was assessed by the 66/68 joint count for swollen and tender joints. Inclusion criteria for patients with PsO were as follows: (i) fulfilled ClASsification of criteria for Psoriatic ARthritis (CASPAR) (see **Table 3.1**); (ii) presence of any clinical variant of PsO; and (iii) patients with PsO were allowed to be on some form of systemic therapy. Corticosteroid usage included cortisone, methylprednisolone, and prednisone. Treatment with synthetic disease-modifying anti-rheumatic drugs (DMARDs) included conventional DMARDs such as leflunomide, methotrexate, and sulfasalazine, as well as the targeted DMARD, upadacitinib (a small-molecule inhibitor). Biologic DMARDs included the TNF inhibitors adalimumab (Humira®), etanercept (Enbrel®), and infliximab (Revellex®). Age-matched healthy individuals with no history of PsO or inflammatory disease were also recruited. WB was collected at the Department of Physiological Sciences, Stellenbosch University, by a Medical Biological Scientist and phlebotomist registered with the Health Professionals Council of South Africa (MW: 0010782). Exclusion criteria for all study participants were as follows: (i) suffering from known chronic inflammatory condition(s), namely human immunodeficiency virus,

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malignancies, and/or tuberculosis; (ii) smoking; (iii) using anticoagulant and/or antiplatelet medication; and (iv) females using contraceptive medication or hormone replacement therapy.

Table 3.1 ClASsification of criteria for Psoriatic ARthritis (Taylor et al., 2006). A patient must have inflammatory articular disease (joint, spine, or entheseal) with a score of ≥3 points of the following five criteria. Current psoriasis is assigned a score of 2, while all other features are assigned a score of 1.

Criterion	Description
1. Evidence of PsO	
Current PsO	Psoriatic skin or scalp disease as examined by a rheumatologist or dermatologist
Personal history of PsO	History of PsO that may be obtained from patient, family physician, dermatologist, rheumatologist, or other healthcare professional
Family history of PsO	History of PsO in first- or second-degree relative according to patient report
2. Psoriatic nail dystrophy	Typical psoriatic nail dystrophy including onycholysis, pitting, and hyperkeratosis observed during current physical examination
3. Negative test for rheumatoid factor	Determined by any method, except latex, but preferably by enzyme-linked immunosorbent assay or nephelometry; according to the local laboratory reference range
4. Dactylitis	
Current dactylitis	Swelling of entire digit
History of dactylitis	History as recorded by a rheumatologist
5. Radiographic evidence of juxta-articular new bone formation	Appearing as ill-defined ossification near joint margins (excluding osteophyte formation) on radiographs of the hand or foot

Abbreviations: PsO, psoriasis.

3.2.3 Collection of Whole Blood and Preparation of Platelet-Poor Plasma

A qualified nurse or phlebotomist collected WB from an antecubital vein via venipuncture, using standard sterile techniques. WB was collected in three 4.5 mL BD Vacutainer® Citrate tubes with 3.2% buffered sodium citrate solution (369714, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Tubes were left at room temperature for at least 30 min before experiments were performed. Sample processing was completed within 24 h of blood collection. Platelet-poor plasma (PPP) was prepared by centrifugation of WB at 3000 x *g* for 15 min at room temperature. Afterwards, PPP was aliquoted and stored at -80°C until further laboratory analysis.

3.2.4 Vascular Injury Panel

The plasma levels of inflammation-related biomarkers were determined using the V-PLEX Vascular Injury Panel 2 (human) Kit (K15198D, Meso Scale Diagnostics [MSD], Rockville, MD, USA). This kit measures four biomarkers associated with acute inflammation and tissue

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damage, namely CRP, serum amyloid A (SAA), soluble ICAM-1 (sICAM-1)/CD54, and soluble VCAM-1 (sVCAM-1)/CD106.

Prior to analysis, PPP from patients with PsO and healthy control subjects was thawed from -80°C to room temperature. PPP was diluted 1000x with the supplied MSD Diluent, as recommended by the manufacturer. Before samples were added, the supplied MSD MULTI-SPOT® 96-Well 4-Spot Plate was washed three times with MSD Wash Buffer. Subsequently, 25 µL of sample, calibrator, or control, was added per well (precoated with capture antibodies) and incubated for 2 h at room temperature. After incubation, the plate was washed again three times with MSD Wash Buffer. Thereafter, 25 µL of detection antibody was added per well and incubated for 1 h at room temperature. Detection antibodies (MSD SULFO-TAG™) are conjugated with electrochemiluminescent labels. The plate was washed one final time with MSD Wash Buffer and 150 µL of MSD Read Buffer was added to each well. The plate was read on the MSD Discovery Workbench 4 instrument by applying a voltage, which causes the emittance of light. The intensity of emitted light is directly proportional to the quantity of analyte present in the sample. The MSD DISCOVERY WORKBENCH® software was used to acquire and analyse data. Samples were analysed in duplicate.

3.2.5 Soluble P-selectin ELISA

The plasma level of sP-selectin/CD62P was determined using the Human sSELP (Soluble P-Selectin) ELISA kit (EH3818, Fine Biotech Co., Ltd., Wuhan, Hubei, China). P-selectin is expressed by activated platelets and endothelial cells. The soluble form has been suggested to originate primarily from activated platelets (Ferroni et al., 2009). Thus, sP-selectin is regarded as a biomarker of *in vivo* platelet activation.

Prior to analysis, PPP from patients with PsO and healthy controls was thawed from -80°C to room temperature. PPP was diluted 250x with the supplied Sample Dilution Buffer. Before samples were added, the supplied ELISA microplate (pre-coated with capture antibodies) was washed two times with the supplied Wash Buffer. Subsequently, 100 µL of sample, standard or control, was added per well and incubated for 1.5 h at 37°C. After incubation, the plate was washed two times with Wash Buffer. Thereafter, 100 µL of Biotin-Labelled Antibody Solution was added per well and incubated at 37°C for 1 h. The plate was washed again three times with Wash Buffer. Subsequently, 100 µL of the Horseradish Peroxidase (HRP)-Streptavidin Conjugate was added per well and incubated for 30 min at 37°C. Streptavidin binds to biotin with high affinity. After washing the plate five times with Wash Buffer, 90 µL of 3,3′,5,5′-Tetramethylbenzidine (TMB) Substrate Solution was added per well and incubated in the dark for 10 min at 37°C. TMB is a colourimetric substrate that reacts with HRP. Finally, 50 µL of

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Stop Solution was added per well, resulting in the formation of a yellow reaction product. The absorbance was read at a wavelength of 450 nm. Samples were analysed in duplicate.

3.2.6 Thromboelastography

Thromboelastography (TEG) is a non-invasive viscoelastometric method to measure the ability of WB to form a clot in a quantitative manner. Briefly, a TEG cup is placed in the TEG analyser and a pin is suspended in the sample from a torsion wire. The cup oscillates around the pin and as coagulation takes place, the cup and pin become 'attached'. Therefore, movement of the cup also transfers motion on the pin. TEG analysis of recalcified citrated samples depends on contact activation (intrinsic coagulation pathway).

Thromboelastography was performed, using the TEG 5000 Hemostasis Analyzer System (07-033, Haemonetics®, Boston, MA, USA), to assess the clot kinetics and viscoelastic properties of naive WB samples from patients with PsO and healthy controls. Samples were prepared as follows: 20 µL of 0.2 M calcium chloride ($CaCl_2$) (7003, Haemonetics®) was added to a disposable TEG cup (6211, Haemonetics®), followed by the addition of 340 µL of WB. $CaCl_2$ was added to reverse the anticoagulant action of sodium citrate and, consequently, activate the coagulation cascade. Samples were loaded into the measuring channels of the TEG instrument and analyses were performed at 37°C. Maintenance checks were performed with each run as prompted by the system. Refer to **Table 3.2** for a brief explanation of the seven TEG parameters that were assessed in this study.

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Table 3.2 Thromboelastography parameters (Srinivasa et al., 2001; Nielsen et al., 2004; Karon, 2014).

Parameter	Unit of measurement	Interpretation
Reaction time (R)	min	**Activation phase** Time from start of test to first detectable fibrin formation (amplitude of 2 mm) Influenced by concentration of coagulation factors
Kinetics (K)	min	**Amplification phase** Time taken to achieve a certain level of clot strength (amplitude of 20 mm) Influenced by fibrinogen concentration and, to a lesser extent, platelet function
Alpha (Angle) (A)	deg	**Propagation phase** The angle measures the maximal speed of thrombin generation and fibrin formation and cross-linking Influenced by fibrinogen concentration and, to a lesser extent, platelet function
Maximum amplitude (MA)	mm	**Termination phase** Maximum mechanical strength/stiffness of clot Influenced by fibrin cross-linking, platelet count, and platelet glycoprotein IIb/IIIa interactions
Maximum rate of thrombus generation (MRTG)	$dyn/cm^2/s$	First derivative of the velocity of the increase in clot strength based on the change in the elastic modulus, G, where G=(5000MA)/(100-MA)
Time to maximum rate of thrombus generation (TMRTG)	min	Time interval observed before maximum speed of clot growth
Total thrombus generation (TTG)	dyn/cm^2	Total area under the velocity curve, representing the clot strength generated during clot growth

3.2.7 Scanning Electron Microscopy

Fibrin clots were prepared from PPP for ultrastructural analysis of the fibrin network architecture by scanning electron microscopy (SEM). To create a fibrin fibre network, 10 µL of PPP was deposited on a 10 mm round glass coverslip and 5 µL of human thrombin (provided by the South African National Blood Service, final concentration 7 IU/mL) was added. Subsequently, samples were covered with 1X Gibco® phosphate-buffered saline (PBS), pH 7.4 (10010015, Thermo Fisher Scientific, Waltham, MA, USA) for at least 15 min. Afterwards, primary fixation was performed by covering samples with 4% formaldehyde (158127, Sigma-Aldrich, St. Louis, MO, USA) for a minimum of 30 min. Thereafter, samples were washed three times for 3 min with PBS. Secondary fixation was performed with 1% osmium tetra-oxide (OsO_4) (75632, Sigma-Aldrich) for 15 min. Afterwards, samples were washed three times for 3 min with PBS. Samples were serially dehydrated with increasing concentrations of ethanol (30%, 50%, 70%, and 90%) and three times with 100% ethanol, for 3 min each. A dehydration step was performed by covering each sample with 99.9% hexamethyldisilazane (HMDS)

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(379212, Sigma-Aldrich) for 30 min. HMDS was removed and a final drop of HMDS was added directly to each sample, whereafter samples were left to air-dry overnight in a flow-hood. Coverslips were mounted with double-sided carbon tape on glass microscope slides and sputter-coated with carbon, using a Quorum Q150T E Plus carbon coater (Quorum Technologies, Laughton, East Sussex, UK). Samples were examined using a Zeiss MERLIN™ Field Emission Scanning Electron Microscope (Carl Zeiss, Oberkochen, Germany). Electron micrographs were captured with the high resolution InLens detector at 1 kV. A minimum of three micrographs were captured for each sample at 4000x and 10 000x magnification, respectively.

Micrographs of a subset of patients with PsO (n=9) and healthy controls (n=9) were identified for further analysis of fibrin fibre diameter using ImageJ (version 1.52a). A grid was overlaid on these micrographs, consisting of five vertical blocks and seven horizontal blocks. Each 1.4 µm x 1.4 µm grid block measured an area of 2 μm^2. In each block of the grid, a fibrin fibre was selected randomly and the fibre diameter measured (**Figure 3.1**). For each of the individuals included in the subset, three micrographs were analysed, with 35 fibres measured per micrograph. Therefore, 105 measurements were taken per individual. In total, 945 measurements were taken in both the patient and control groups. In order to graphically illustrate differences in the distribution of fibrin fibre diameter, in healthy controls and patients with PsO, frequency bar graphs were constructed. In addition, differences in the minimum, maximum, and mean fibre diameters were evaluated between groups.

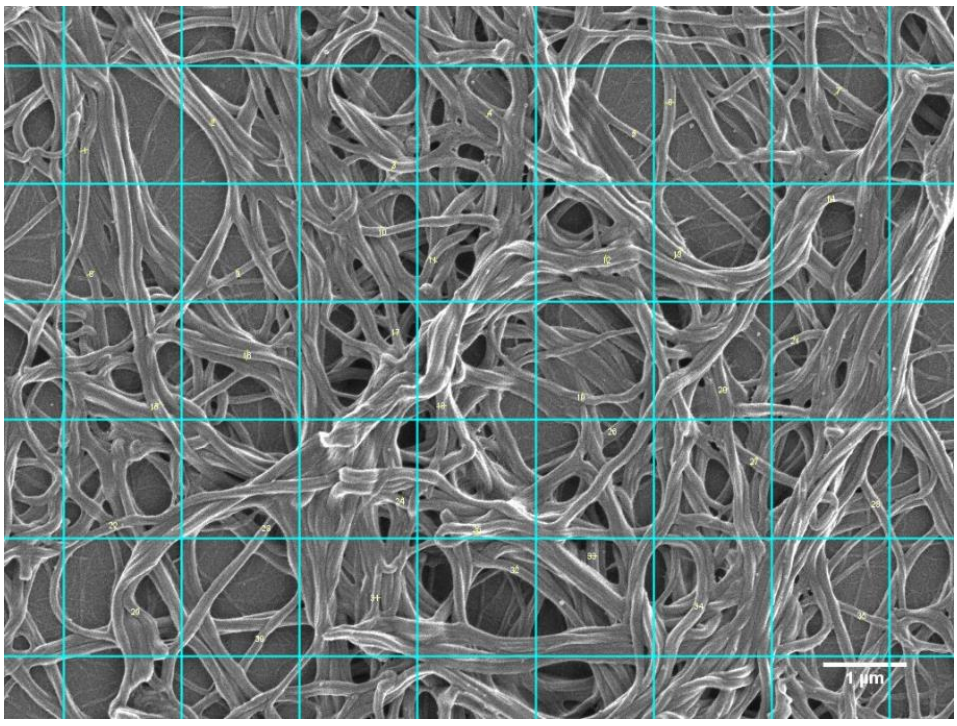

Figure 3.1 Representative scanning electron micrograph with grid overlay. Numbered fibres indicate fibres that were measured. Individual grid blocks represent an area of 2 μm^2. Total grid area is 97 μm^2 (scale bar=1 µm).

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3.2.8 Statistical Analysis

GraphPad Prism version 8.4.3 software was used to produce summaries and plots of the data. In order to summarise differences between groups, an unpaired student's t-test was performed for normally distributed data (as determined by the Shapiro–Wilk normality test), while the Mann–Whitney *U* test was performed for non-normally distributed data. Normally distributed data are expressed as mean ± standard error of the mean and non-normally distributed data are expressed as median and (25–75% quartile range). R version 4.0.3 software was used to perform logistic regression to determine the strength of associations between study variables and disease status (presence or absence of PsO). More specifically, logistic regression was performed on covariates directly (model 1) and with adjustment for age and gender (model 2). Odds ratios (ORs) are reported with 95% confidence intervals (CIs). A *p*-value of less than 0.05 was considered statistically significant. A single missing TEG K value of a healthy control subject was imputed using the mean of the K values of the control population.

3.3 Results

The demographic features and clinical characteristics of healthy individuals and patients with PsO are presented in **Table 3.3**. There were no significant differences in age (p=0.274) and gender (p=0.741) between the study groups. According to DAPSA scores, four patients presented with low disease activity, seven with moderate disease activity, and nine with high disease activity. Dactylitis was present in seven patients. The median PASI score was 5.300, representing moderate disease severity.

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Table 3.3 Demographic features and clinical characteristics of healthy individuals and patients with psoriasis. Normally distributed data are expressed as mean ± standard error of the mean and non-normally distributed data are expressed as median and (25-75% quartile range).

Demographics	**Healthy individuals (n=20)**	**Patients with PsO (n=20)**
Age, years	58.150 ± 2.785	54.250 ± 2.148
Gender		
Female, n (%)	6 (30)	8 (40)
Male, n (%)	14 (70)	12 (60)
Comorbidities		
Anaemia, n (%)		1 (5)
Diabetes, n (%)		5 (25)
Hypercholesterolaemia, n (%)		4 (20)
Hypertension, n (%)		9 (45)
Hypothyroidism, n (%)		2 (10)
Ischaemic heart disease, n (%)		3 (15)
Menopause, n (%)		1 (5)
Disease activity		
DAPSA		
Low, n (%)		4 (20)
Moderate, n (%)		7 (35)
High, n (%)		9 (45)
Dactylitis, n (%)		7 (35)
PASI		5.300 (1.075–14.850)
Treatment		
None		5 (25)
DMARD, n (%)		9 (45)
Biologic agent, n (%)		2 (10)
Topical corticosteroid + DMARD, n (%)		2 (10)
Topical corticosteroid + Biologic agent, n (%)		1 (5)
Biologic agent + DMARD, n (%)		1 (5)

Abbreviations: DAPSA, Disease Activity in PSoriatic Arthritis; DMARD, disease-modifying anti-rheumatic drug; PASI, Psoriasis Area and Severity Index; PsO, psoriasis.

Biomarker analysis was performed to determine the levels of specific inflammatory molecules in the PPP of healthy individuals and patients with PsO. Box-and-whisker plots, showing the distribution of these parameters in healthy individuals and individuals with PsO, are shown in **Figure 3.2**. CRP, SAA, and sICAM-1 levels were significantly elevated in patients with PsO when compared to controls (**Figure 3.2**). No significant differences were noted in the sVCAM-1 levels when individuals with PsO were compared to control subjects. Logistic regression models indicated that elevated levels of acute-phase reactants, namely CRP (OR 1.402, CI 1.146–1.838) and SAA (OR 1.144, CI 1.038–1.315), were significantly associated with the presence of PsO (**Table 3.4**). Elevated concentrations of these biomarkers indicate the presence of inflammation in patients with PsO. In addition, sICAM-1 (OR 1.012, CI 1.003–1.022) was another marker significantly associated with PsO (**Table 3.4**). Increased levels of sICAM-1 reflect endothelial cell activation. After adjusting for the effects of age and gender, higher sP-selectin (OR 1.101, CI 1.005–1.228) levels were significantly associated with disease presence (**Table 3.4**). An elevated concentration of sP-selectin indicates platelet activation, as this adhesion molecule is expressed on the platelet surface upon activation.

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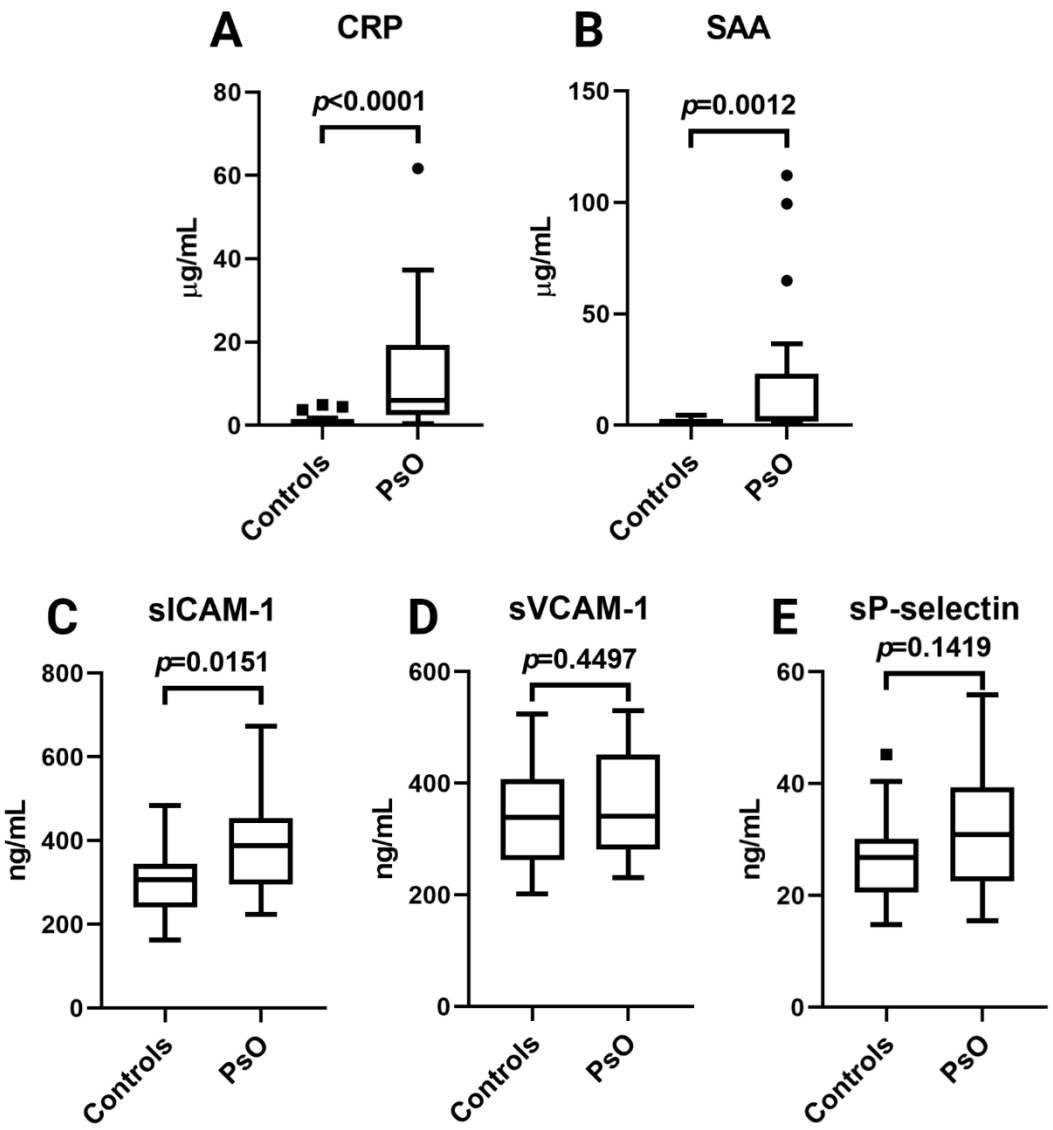

Figure 3.2 Box-and-whisker plots illustrating the distribution of laboratory parameters assessed in healthy individuals and patients with psoriasis. Box-and-whisker plots were produced using GraphPad Prism version 8.4.3 software. **Abbreviations:** CRP, C-reactive protein; PsO, psoriasis; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sP-selectin, soluble P-selectin; sVCAM-1, soluble vascular cell adhesion molecule-1.

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Table 3.4 Results of logistic regression on laboratory parameters in healthy individuals and patients with psoriasis. Data are expressed as median and (25–75% quartile range). Odds ratios are reported at 95% confidence intervals. Asterisks indicate statistically significant associations.

Inflammatory markers	**Healthy individuals (n=20)**	**Patients with PsO (n=20)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
CRP (µg/mL)	0.570 (0.295–1.508)	6.015 (2.565–19.082)	1.375 (1.145–1.724)*	1.402 (1.146–1.838)*
SAA (µg/mL)	1.110 (0.590–2.615)	2.660 (2.020–15.332)	1.145 (1.034–1.320)*	1.144 (1.038–1.315)*
sICAM-1 (ng/mL)	306.150 (270.360–342.470)	388.480 (314.750–449.470)	1.009 (1.002–1.019)*	1.012 (1.003–1.022)*
sVCAM-1 (ng/mL)	338.940 (279.420–400.590)	340.930 (297.680–449.790)	1.003 (0.996–1.011)	1.005 (0.997–1.014)
Platelet activation marker	**Healthy individuals (n=15)**	**Patients with PsO (n=20)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
sP-selectin (ng/mL)	26.780 (22.825–29.340)	30.860 (23.873–39.013)	1.064 (0.987–1.159)	1.101 (1.005–1.228)*

Abbreviations: CI, confidence interval; CRP, C-reactive protein; OR, odds ratio; PsO, psoriasis; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sP-selectin, soluble P-selectin; sVCAM-1, soluble vascular cell adhesion molecule-1.

TEG analysis was performed on WB samples from healthy controls and patients with PsO to assess coagulation status. Seven WB clot parameters were assessed in this study (**Table 3.2**) and the distribution of TEG parameters is illustrated by box-and-whisker plots in **Figure 3.3**. Significant differences were detected in five TEG parameters (**Figure 3.3** and **Table 3.5**). Logistic regression modelling showed that shortened reaction time (R) (OR 0.682, CI 0.497–0.877) and kinetics (K) (OR 0.384, CI 0.169–0.733) values were significantly associated with PsO. Accelerated fibrin cross-linking, indicated by an increase in Alpha (A) (OR 1.157, CI 1.043–1.302), was also identified as a significant parameter. Moreover, after adjustment, an increase in the maximum rate of thrombus generation (MRTG) (OR 1.637, CI 1.046–2.894) was also significantly associated with PsO. Furthermore, a significant association was also detected between a shortened time to maximum rate of thrombus generation (TMRTG) (OR 0.778, CI 0.636–0.922) value and disease presence. Altogether, these results indicate that an altered coagulation profile, characterised by an increased tendency to form a blood clot, is associated with PsO.

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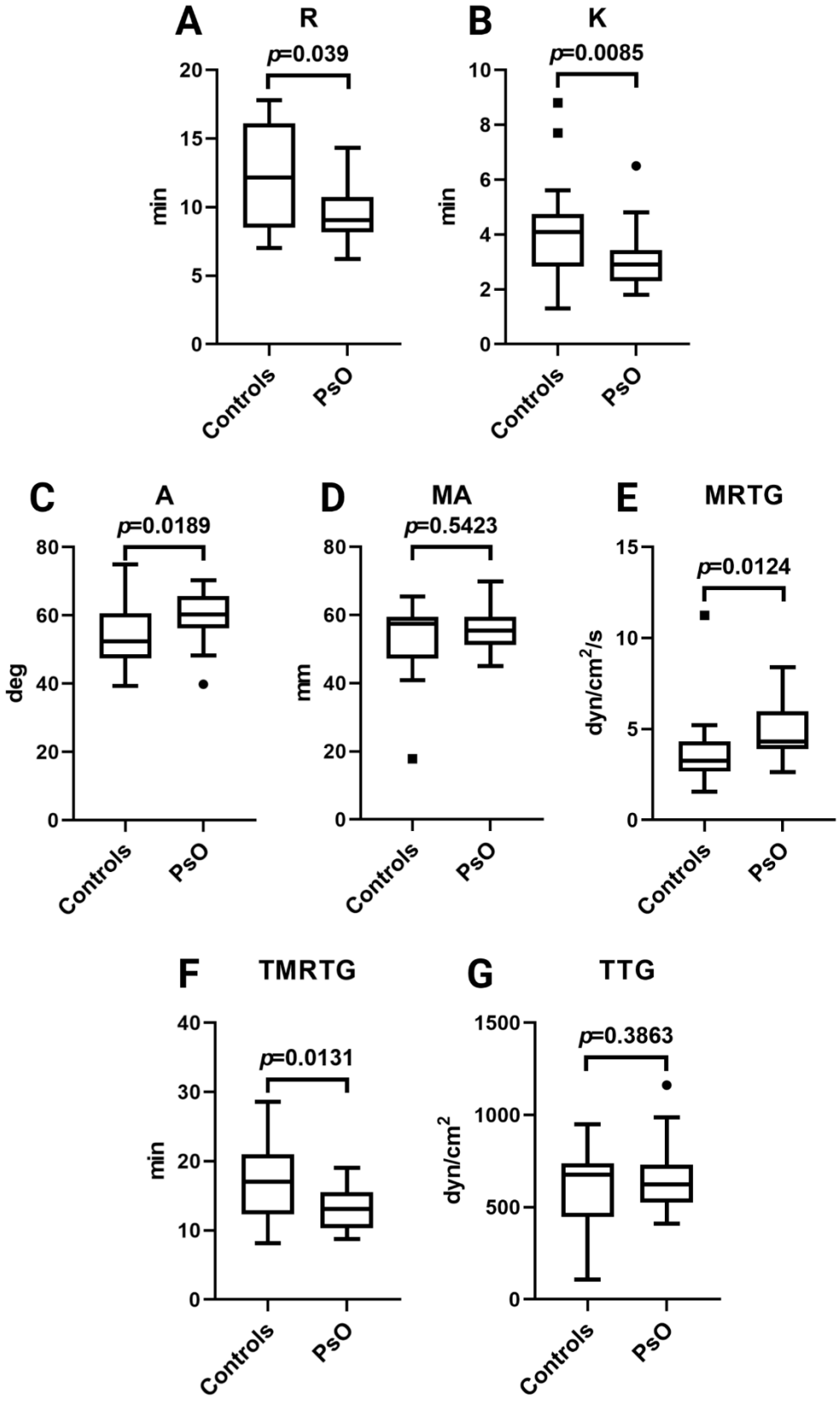

Figure 3.3 Box-and-whisker plots illustrating the distribution of thromboelastography parameters assessed in healthy individuals and patients with psoriasis. Box-and-whisker plots were produced using GraphPad Prism version 8.4.3 software. **Abbreviations:** A, Alpha (Angle); K, kinetics; MA, maximum amplitude; MRTG, maximum rate of thrombus generation; PsO, psoriasis; R, reaction time; TMRTG, time to maximum rate of thrombus generation; TTG, total thrombus generation.

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Table 3.5 Results of logistic regression on thromboelastography whole blood coagulation parameters in healthy individuals and patients with psoriasis. Data are expressed as median and (25–75% quartile range). Odds ratios are reported at 95% confidence intervals. Asterisks indicate statistically significant associations.

TEG WB coagulation parameter	Healthy individuals (n=20)	Patients with PsO (n=20)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
R (min)	12.150 (8.700–15.975)	9.050 (8.250–10.575)	0.732 (0.555–0.919)*	0.682 (0.497–0.877)*
K (min)	4.095 (2.875–4.650)	2.900 (2.500–3.275)	0.490 (0.242–0.816)*	0.384 (0.169–0.733)*
A (deg)	52.450 (48.050–60.525)	60.250 (57.325–63.825)	1.112 (1.021–1.224)*	1.157 (1.043–1.302)*
MA (mm)	57.450 (49.125–58.900)	55.400 (52.250–59.400)	1.048 (0.976–1.145)	1.055 (0.969–1.178)
MRTG (dyn/cm^2/s)	3.255 (2.758–4.303)	4.315 (3.918–5.963)	1.530 (0.993–2.537)	1.637 (1.046–2.894)*
TMRTG (min)	17.050 (12.397–20.940)	13.125 (10.397–15.520)	0.821 (0.677–0.954)*	0.778 (0.636–0.922)*
TTG (dyn/cm^2)	676.950 (483.790–719.400)	623.840 (547.410–717.960)	1.002 (0.998–1.005)	1.002 (0.998–1.006)

Abbreviations: A, Alpha (Angle); CI, confidence interval; K, kinetics; MA, maximum amplitude; MRTG, maximum rate of thrombus generation; OR, odds ratio; PsO, psoriasis; R, reaction time; TEG, thromboelastography; TMRGT, time to maximum rate of thrombus generation; TTG, total thrombus generation; WB, whole blood.

The fibrin network architecture may influence clot properties; therefore, SEM was utilised to detect differences in the ultrastructure of fibrin fibre networks (PPP clots) from healthy individuals and individuals with PsO. Representative scanning electron micrographs of PPP clots from healthy control subjects and patients with PsO are depicted in **Figure 3.4** and **Figure 3.5**, respectively. Typically, fibrin networks from healthy individuals (shown in **Figure 3.4**) appeared as ‘loose’ networks of fibrin fibres. Individual fibres were clearly discernible, and pores were observed regularly between fibres. In contrast, fibrin networks of individuals with PsO seemed to be more compact (**Figure 3.5**). Individual fibrin fibres could not always be distinguished and multiple fibres were fused (indicated by the boxes in **Figure 3.5A** and **F**).

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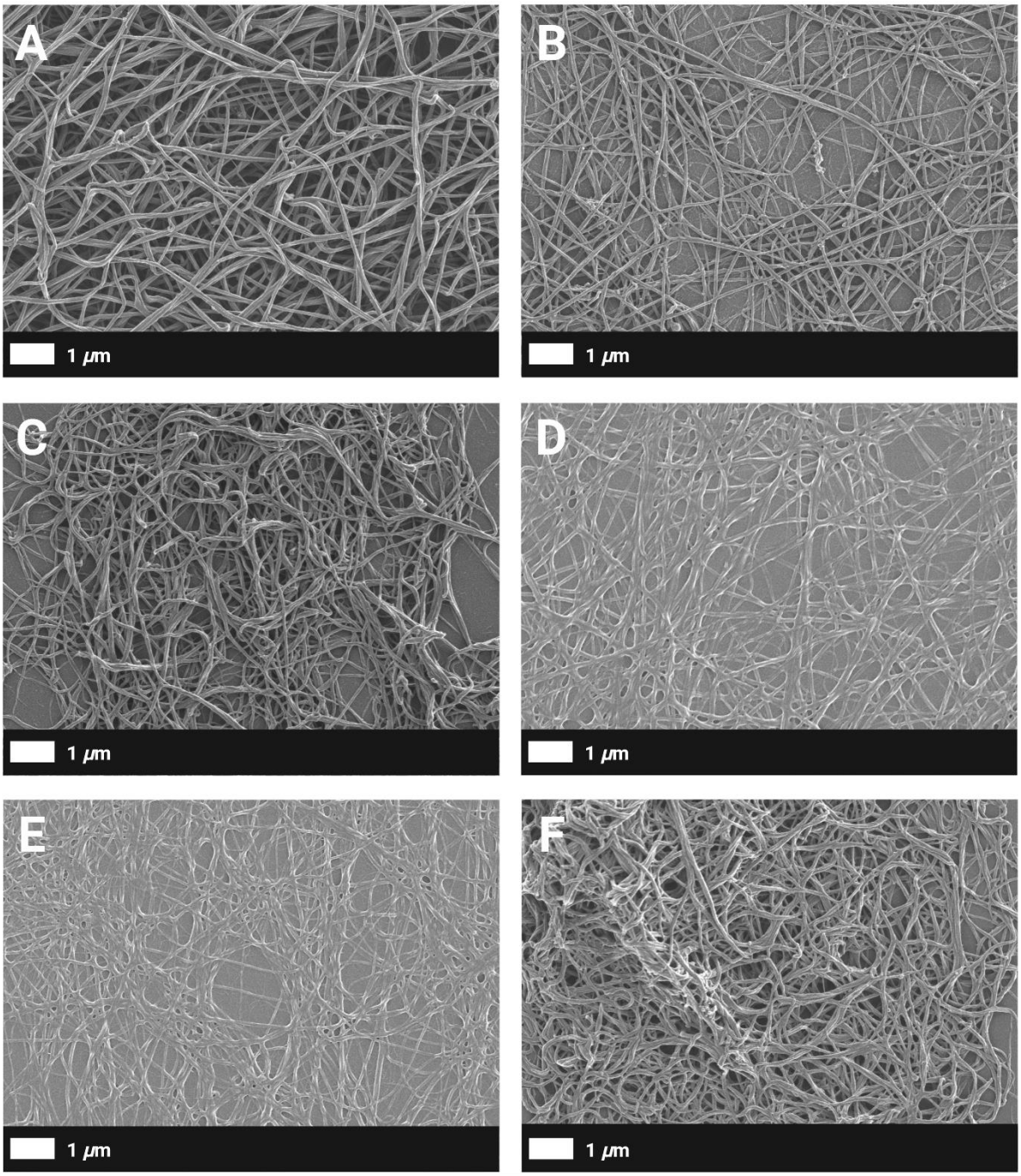

Figure 3.4 Representative scanning electron micrographs of fibrin networks (prepared from platelet-poor plasma) from healthy individuals. (**A**) A fibrin network of a 47-year-old female individual. (**B**) A fibrin network of a 50-year-old male individual. (**C**) A fibrin network of a 53-year-old male individual. (**D**) A fibrin network of a 35-year-old male individual. (**E**) A fibrin network of a 47-year-old male individual. (**F**) A fibrin network of a 60-year-old male individual.

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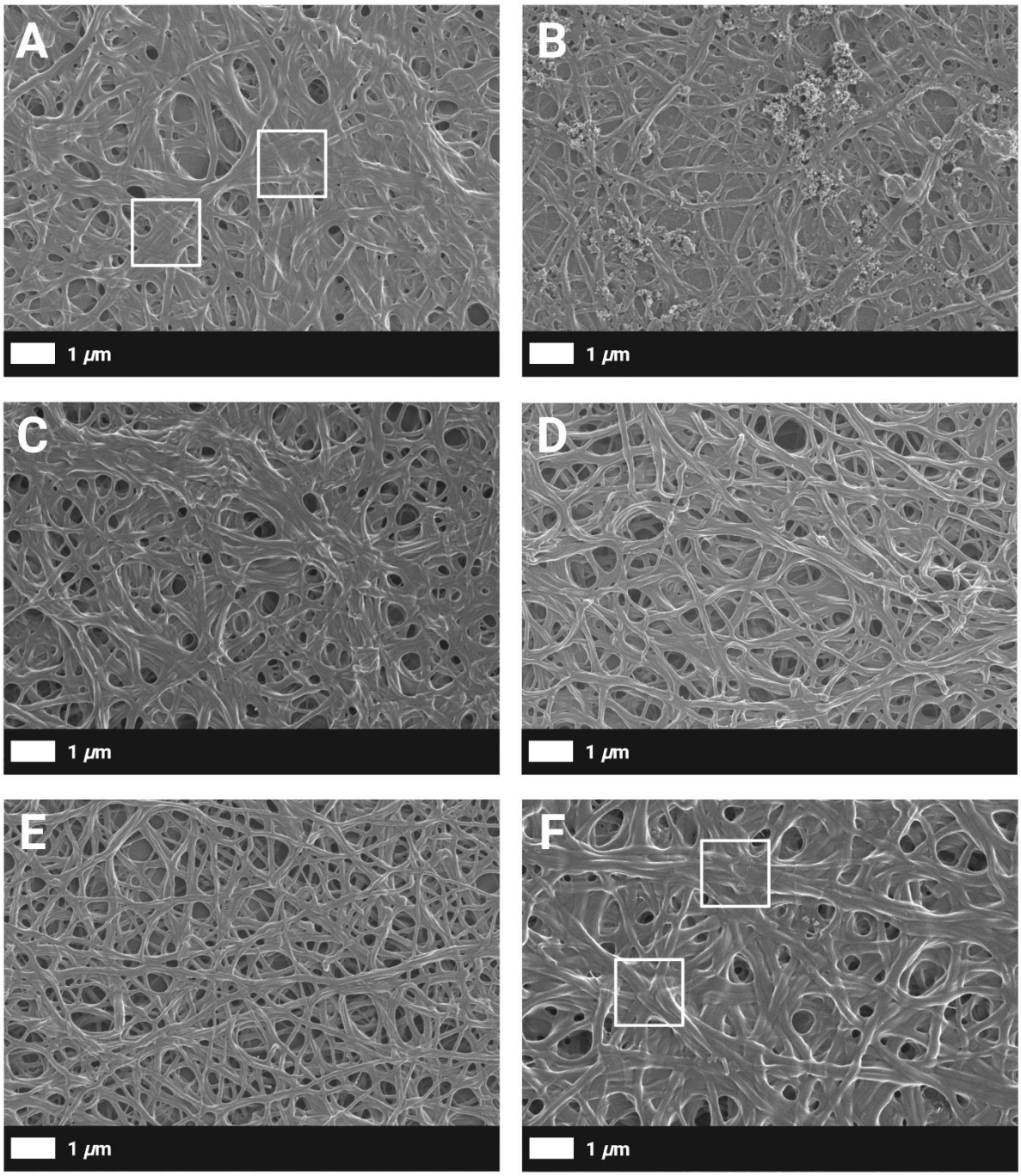

Figure 3.5 Representative scanning electron micrographs of fibrin networks (prepared form platelet-poor plasma) from patients with psoriasis. Patients did not receive any treatment unless stated otherwise. (**A**) A fibrin network of a 45-year-old female individual with low psoriatic arthritis (PsA) disease activity and mild skin involvement (**B**) A fibrin network of a 50-year-old male individual with high PsA disease activity and mild skin involvement. (**C**) A fibrin network of a 44-year-old male individual with moderate PsA disease activity and mild skin involvement. This patient was treated with a disease-modifying anti-rheumatic drug (DMARD). (**D**) A fibrin network of a 57-year-old male individual with moderate PsA disease activity and moderate skin involvement. This patient was treated with a DMARD. (**E**) A fibrin network of a 49-year-old male individual with high PsA disease activity and moderate skin involvement. (**F**) A fibrin network of a 34-year-old female individual with high PsA disease activity and severe skin involvement.

Fibrin fibre diameter was measured in a subset of healthy individuals and individuals with PsO. Patients with PsO included in the subset were chosen to represent low, moderate, and high disease activity of PsA (according to DAPSA scores), with mild to severe skin involvement

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(according to PASI scores), to ensure that disease activity of both the joint and skin domains was taken into account. The mean, minimum, and maximum fibre diameters were determined for each individual in both the control and patient groups. For the minimum and maximum fibre diameters, the smallest and largest measurements of an individual were grouped as follows: minimum of healthy individuals, maximum of healthy individuals, minimum of patients with PsO, and maximum of patients with PsO. **Figure 3.6** shows box-and-whisker plots illustrating the distribution of these variables in the control and study populations.

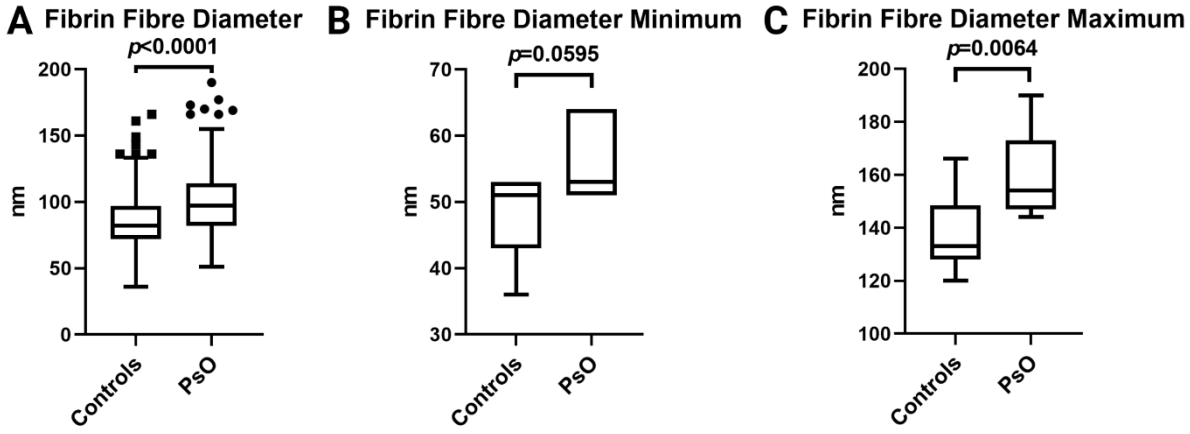

Figure 3.6 Box-and-whisker plots illustrating the distribution of the mean, minimum, and maximum fibrin fibre diameter in healthy individuals and patients with psoriasis. Box-and-whisker plots were produced using GraphPad Prism version 8.4.3 software. **Abbreviations:** PsO, psoriasis.

Frequency bar graphs were constructed (**Figure 3.7**) to graphically illustrate differences between the distribution of fibrin fibre diameter in healthy individuals and patients with PsO. In comparison to healthy controls, the fibrin fibre diameter was significantly increased in individuals with PsO (**Figure 3.6**). This was also reflected by the frequency bar graphs that showed greater counts of thicker fibres in patients with PsO (**Figure 3.7B**). Logistic regression analysis showed that an increased fibrin fibre diameter (OR 1.562, CI 1.234–2.173) was associated with disease presence (**Table 3.6**). Similarly, increases in the minimum (OR 1.483, CI 1.126–2.225) and maximum (OR 1.121, CI 1.041–1.240) fibrin fibre diameters were also associated with the presence of PsO (**Table 3.6**).

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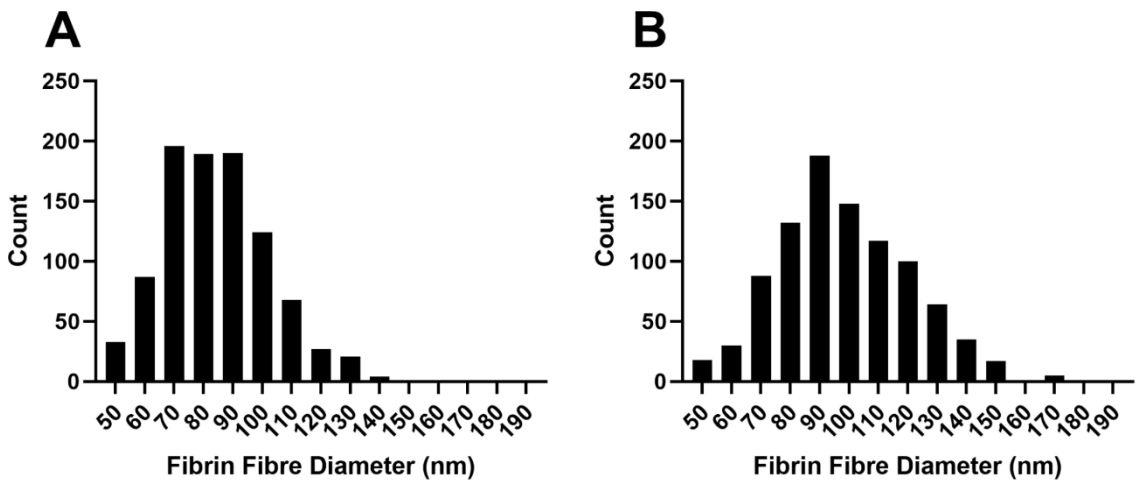

Figure 3.7 Frequency bar graphs of fibrin fibre diameter distribution. (**A**) Fibre diameter distribution in healthy individuals. (**B**) Fibre diameter distribution in patients with psoriasis. Bar graphs were produced using GraphPad Prism version 8.4.3 software.

Table 3.6 Results of logistic regression on fibrin fibre diameter in healthy individuals and patients with psoriasis. Data are expressed as median and (25–75% quartile range). Odds ratios are reported at 95% confidence intervals. Asterisks indicate statistically significant differences.

Fibrin fibre diameter	Healthy individuals (n=9)	Patients with PsO (n=9)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Mean (nm)	83.724 (83.390–86.552)	99.043 (93.336–106.120)	1.470 (1.172–2.014)*	1.562 (1.234–2.173)*
Minimum mean (nm)	51.000 (46.000–53.000)	53.000 (51.000–62.250)	1.410 (1.107–2.062)*	1.483 (1.126–2.225)*
Maximum mean (nm)	133.000 (130.000–136.000)	160.000 (150.000–168.250)	1.112 (1.036–1.230)*	1.121 (1.041–1.240)*

Abbreviations: CI, confidence interval; OR, odds ratio; PsO, psoriasis.

3.4 Discussion

PsO is a T cell-mediated chronic inflammatory skin disease characterised by the hyperproliferation of keratinocytes. However, several extracutaneous manifestations have also been linked to the prevalence of the condition (Grozdev et al., 2014; Korman, 2020). In particular, an increased burden of cardiovascular morbidity and mortality has been observed in individuals with PsO. Nevertheless, the causes of CVD prevalence in psoriatic disease have not been fully elucidated. In this study, it was shown that patients with PsO presented with a biomarker profile that reflected systemic inflammation, endothelial activation, and heightened platelet activity. Moreover, altered viscoelastic properties of WB and structural changes in the fibrin network architecture were observed in these individuals. An illustrative summary of these results is provided in **Figure 3.8**.

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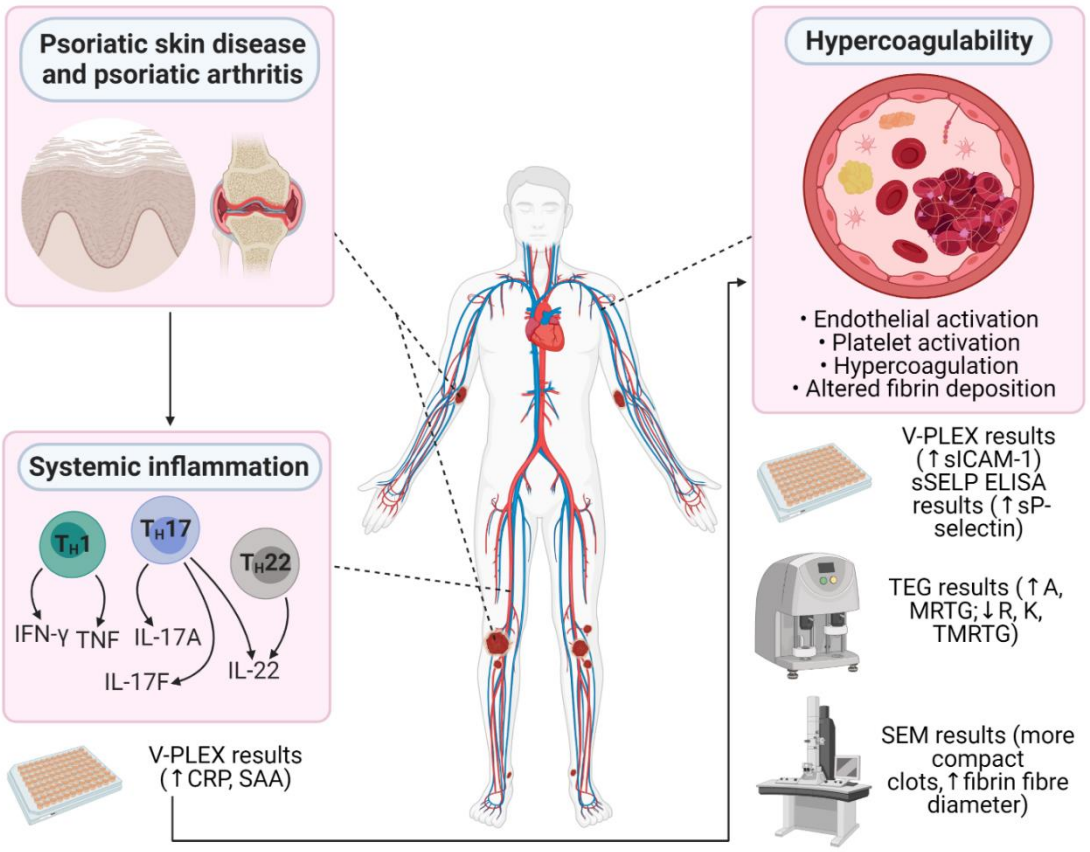

Figure 3.8 A summary of the key findings of this study. Various pro-inflammatory molecules are implicated in the onset and maintenance of psoriasis (PsO). These inflammatory mediators may potentially spill over into circulation, resulting in systemic inflammation. Accordingly, patients in this study presented with elevated levels of acute-phase reactants (C-reactive protein and serum amyloid A). As the processes of inflammation and coagulation are interconnected, persistent systemic inflammation may promote the development of a prothrombotic state in individuals living with PsO. In this study, the prothrombotic state in patients with PsO was characterised by endothelial (elevated soluble intercellular adhesion molecule-1 levels) and platelet (elevated soluble P-selectin levels) activation, an increased tendency towards clot formation (altered thromboelastography parameters), and abnormal fibrin deposition (scanning electron microscopy analysis). Diagram created with BioRender.com. **Abbreviations:** A, Alpha (Angle); CRP, C-reactive protein; IFN-γ, interferon-γ; IL, interleukin; K, kinetics; MRTG, maximum rate of thrombus generation; R, reaction time; SAA, serum amyloid A; SEM, scanning electron microscopy; sICAM-1, soluble intercellular adhesion molecule-1; sP-selectin, soluble P-selectin; TEG, thromboelastography; T_H, T helper; TMRTG, time to maximum rate of thrombus generation; TNF, tumour necrosis factor.

The presence of a peripheral inflammatory milieu in patients with PsO was confirmed by the association between increased levels of CRP and SAA, and the condition (**Table 3.4**). These proteins are acute-phase reactants that are hepatically synthesised and released into circulation in response to inflammation and/or tissue injury. TNF, a major mediator of PsO pathogenesis, has been shown to induce the expression of IL-6. The latter, which is also produced by keratinocytes in psoriatic skin lesions (Grossman et al., 1989) and the inflamed synovium in PsA (van Kuijk et al., 2006), stimulates the synthesis of CRP. Therefore, elevated CRP levels may reflect the participation of pro-inflammatory cytokines in the psoriatic disease process. SAA has been shown to be overexpressed in lesional skin of patients with PsO (Couderc et al., 2017) and may also be detected in the inflamed synovial tissue of patients with

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PsA (O'Hara et al., 2004). Both CRP and SAA possess procoagulant activity and may contribute to a hypercoagulable state via the induction of TF in endothelial cells and monocytes, as well as the suppression of anticoagulant activity (Cermak et al., 1993; Singh et al., 2005; Cai et al., 2007; Zhao et al., 2007; Chen et al., 2009). Furthermore, endothelial cell activation, indicated by raised sICAM-1 levels, was also associated, albeit weakly, with the presence of disease (**Table 3.4**). Membrane-bound ICAM-1 is involved in leukocyte extravasation to sites of inflammation (Schnoor et al., 2015) and the overexpression of this CAM has been demonstrated in both lesional and non-lesional skin of individuals with PsO (Rottman et al., 2001). ICAM-1 may promote thrombus formation by facilitating the adhesion of activated platelets to the endothelium (Bombeli et al., 1998; Tsakadze et al., 2002). One may speculate that the increased plasma levels of the aforementioned molecules are due to a 'spill-over' effect from affected skin areas, reinforcing peripheral inflammation.

Limited studies have investigated the coagulation profiles of individuals with PsO. WB coagulation was assessed using TEG as it provides a more comprehensive view of haemostasis compared to conventional laboratory tests (Srinivasa et al., 2001). Shortened values of parameters that measure clot formation initiation and amplification (R and K) were associated with the presence of disease (**Table 3.5**). Enhanced clot propagation (A, MRTG, and TMRTG) was also related to the presence of PsO (**Table 3.5**). These changes may be attributed to elevated levels of fibrinogen or an increased rate of thrombin generation. Fibrinogen levels were not measured in this study; however, other studies have reported raised levels in patients with PsO (Rocha-Pereira et al., 2004; Kimhi et al., 2007; Arias-Santiago et al., 2012). Pro-inflammatory molecules, which circulate at elevated levels in active PsO, may induce TF expression by vascular endothelial cells and peripheral blood monocytes (Neumann et al., 1997) while simultaneously suppressing the activity of thrombomodulin (Hot et al., 2012), leading to rapid thrombin generation and fibrin deposition.

Despite an increased tendency to form a clot, parameters related to clot strength (MA and TTG) appeared unaltered in individuals with PsO compared to healthy individuals (**Table 3.5**). This finding was likely caused by compromised fibrin network structure and/or platelet function in patients with PsO. It should be noted that several patients that were included in this study received some form of immunosuppressant therapy (**Table 3.3**), which may be a confounding factor. Methotrexate and biological agents may potentially cause thrombocytopenia (Paul et al., 2015). On the contrary, various studies have reported that platelets are in an activated state in patients with PsO (Canpolat et al., 2010; Tamagawa-Mineoka et al., 2010). In this study, platelets did appear to be activated as elevated sP-selectin levels were associated with PsO (**Table 3.4**). It has been demonstrated that platelet contraction results in a concomitant increase in clot stiffness through rearrangement of the fibrin network (Kim et al., 2017). Importantly, refractoriness of platelets (as a result of hyperactivation) may decrease their ability

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to participate in the process of clot contraction, which may also influence the outcome of an occlusive thrombus. Tutwiler and colleagues (2017) have reported impaired clot contraction in acute ischaemic stroke patients, suggesting that this may lead to a greater reduction in intravascular blood flow. A more recent study, by the same group, also noted that diminished clot contraction may be predictive of embolisation in pathological states (Tutwiler et al., 2020).

Altered fibrin network architecture has been linked to the development of thrombotic disease (Ząbczyk and Undas, 2017); therefore, plasma clot ultrastructure was assessed with SEM. According to our knowledge, this is the first study in which the fibrin network structure in patients with PsO has been investigated. We have found that the fibrin network of individuals with PsO appeared to be denser with multiple fused fibres (**Figure 3.5**), compared to those of healthy individuals. An increased fibrin fibre diameter (across all groups) was also more prevalent in patients with PsO (**Table 3.6**). It has been reported that denser clots composed of thin fibrin fibres are more resistant to fibrinolytic degradation than clots composed of thick fibres (Collet et al., 2006). However, it should be borne in mind that various factors, such as the concentration of coagulation factors [e.g., thrombin (Wolberg, 2007)], cellular interactions [e.g., neutrophil extracellular trap formation (Varjú et al., 2015)], and post-translational modifications [e.g., oxidation (Becatti et al., 2014)], may determine final clot structure. The altered structural properties of fibrin clots along with an increased propensity to form a clot (as indicated by TEG results), may confer an increased risk of thrombosis in patients with PsO. In addition, these patients may potentially be more susceptible to embolisation, as platelet function seems to be impaired either due to hyperactivation or a secondary effect of systemic therapy.

This study has limitations. First, this study is cross-sectional in nature; therefore, no inferences can be made with regard to causality. Second, the number of study participants was limited. Finally, patients with PsO receiving systemic treatment were included in this study, which may have a potentially confounding effect.

3.5 Conclusion

PsO is associated with an increased risk of developing CVD. Here, it was confirmed that circulating inflammatory molecules were significantly increased in the disease population. These results, therefore, add to the accumulating evidence of the systemic inflammatory nature of PsO and the subsequent risk of cardiovascular comorbidities. It was also shown that patients with PsO presented with an altered coagulation profile, characterised by an inclination towards clot formation. In addition, it has been demonstrated for the first time, that denser fibrin networks composed of thick fibrin fibres are formed in individuals with PsO. These changes might have implications for the outcome of thromboembolic complications in the context of PsO. Prospective cohort studies should be conducted to confirm the findings of this study.

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Furthermore, remaining uncertainties such as determining the lytic susceptibility of an altered clot structure in PsO should also be addressed.

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Chapter 4: Characterising Fibrin Secondary Structure in Psoriasis: A Combined Qualitative and Quantitative Approach

Abstract

Individuals with psoriasis (PsO) are at an increased risk of developing major adverse cardiovascular events. Fibrinogen plays a key role in haemostasis, and aberrant fibrin clot structure has been linked to thrombotic disorders. The mechanical properties of a clot may contribute to clot stability and, therefore, determine the outcome of an occlusive thrombus to some degree. Under specific conditions, fibrin may undergo a secondary structure transition, characterised by the conversion of α-helices to β-sheets, which influences its mechanical features. It has also been suggested that this transition may occur under pro-inflammatory conditions. Fibrin secondary structure was assessed by examining formalin-fixed, paraffin-embedded clot sections (prepared from the platelet-poor plasma of 20 patients with psoriatic disease and 10 healthy control subjects) using fluorescence microscopy and Fourier transform infrared (FTIR) spectroscopy. Congo red staining was performed to detect β-sheet-rich areas in fibrin clots, and sections were examined by brightfield and fluorescence microscopy. FTIR analysis was utilised to ascertain if there were any quantitative differences in the proportional distribution of the specific secondary structures of fibrin clots. Congo red fluorescence was detected in fibrin clots of healthy control subjects and patients with PsO. No significant differences were observed in the fibrin content of specific secondary structure elements, when clots from patients with PsO were compared to those from healthy control subjects.

4.1 Introduction

Multiple epidemiological studies have reported an elevated risk of arterial and venous occlusive diseases in individuals living with psoriasis (PsO), when compared to the general population. A possible mechanism contributing to this relationship may be the disruption of haemostasis to favour coagulation. Thrombosis is the main cause of cardiovascular complications. A prothrombotic state in patients with PsO may be driven by chronic inflammation (see **Chapter 2**). Elevated levels of pro-inflammatory molecules present in the circulation of individuals with PsO may induce cellular expression of tissue factor (TF), activate platelets and/or augment the platelet response, and perturb the vascular endothelium. Taken together, these perturbations will promote the initiation of coagulation. In addition, the inflammatory milieu may also influence – albeit indirectly – fibrin clot structure and, by extension, the properties of the fibrin network.

Fibrinogen is a 340 kDa glycoprotein composed of three pairs of polypeptide chains, namely Aα, Bβ, and γ, which are stabilised by 29 disulphide bonds. The molecule has a trinodular structure that comprises a central globular E region and two distal globular D regions

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connected by three-stranded α-helical coiled-coil segments. Fibrinogen is a highly abundant plasma protein and plays a pivotal role in various biological processes, such as haemostasis, inflammation, wound healing, and angiogenesis. In case of injury to the vascular endothelium, the activation of the coagulation cascade ensues. This pathway culminates in the generation of large quantities of thrombin, which catalyses the conversion of soluble fibrinogen to insoluble fibrin. During fibrin polymerisation, thrombin cleaves off two fibrinopeptides A and two fibrinopeptides B from the Aα and Bβ chains, resulting in the generation of fibrin monomers. The release of fibrinopeptides exposes binding sites, termed 'knobs', in the central region of fibrinogen molecules. These sites are complementary to sites that are exposed at the C-terminal ends of the molecule, termed 'holes'. The polymerisation of monomers, via knob–hole interactions, gives rise to half-staggered double-stranded protofibrils. The lateral and longitudinal aggregation of protofibrils results in the formation of fibrin fibres that branch to form a three-dimensional filamentous network. Finally, the transglutaminase, factor XIII, which is also activated by thrombin, catalyses the covalent cross-linking of fibrin fibres. This cross-linking renders the clot more stable and resistant to fibrinolytic dissolution. Fibrin formation is closely regulated by several inhibitory mechanisms, specifically the fibrinolytic system (Cesarman-Maus and Hajjar, 2005). The primary fibrinolytic protease, plasmin, is generated when its zymogen form, plasminogen, is converted by tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator. Briefly, plasminogen and tPA bind to the fibrin clot surface, generating plasmin that cleaves fibrin at distinct sites, yielding specific fibrin degradation products.

The fibrin network architecture has been related to thrombotic disease (Ariëns, 2013). A prothrombotic clot phenotype, characterised by densely packed thin fibrin fibres (Collet et al., 2006; Bridge et al., 2014), has been associated with a number of cardiovascular disorders (Undas and Ariëns, 2011). These thrombogenic clots are more resistant to lysis, potentially due to the interplay between fibrin network conformation and the movement of fibrinolytic agents (Collet et al., 2000). A variety of physiological factors, such as the concentration of fibrinogen and (pro)thrombin, the activity of factor XIII, cellular procoagulant activity, and blood flow, may modulate clot structure. An elevated prothrombin concentration has been shown to increase the rate and amount of thrombin generated, resulting in the formation of a fibrin clot composed of thin fibres (Wolberg et al., 2003). Furthermore, endothelial cells stimulated by pro-inflammatory cytokines have been shown to exhibit an increase in procoagulant activity (mediated by an increase in TF activity) and produce an abnormal fibrin network more resistant to lysis (Campbell et al., 2009). Blood flow is another factor that may impact clot structure, and it has been reported that fibrin fibres align along the flow direction (Gersh et al., 2010).

In addition to the fibrin network architecture, the mechanical properties of a clot may also contribute to the pathology of thrombosis. A clot that is very stiff may be more prone to

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embolisation, whereas a more viscous clot may be more easily deformed (Weisel, 2007). Fibrin is a viscoelastic biopolymer; therefore it has both elastic and viscous properties. Clot stiffness (the elastic component) seems to originate from the bending of individual fibres (Collet et al., 2005), whereas the viscosity (the inelastic component) appears to arise from the slippage of protofibrils past one another (Münster et al., 2013). Fibrin exhibits non-linear elasticity, meaning that with small strains (or deformations) the stiffness is proportional to the strain, whereas with larger strains the stiffness of the clot increases dramatically. This behaviour is known as strain-stiffening and appears to depend on the fraction of β-sheets that are present. Single-molecule force spectroscopy experiments have shown that β-sheets are more resistant to mechanical unfolding, compared to α-helices (Rief et al., 1997; Rief et al., 1999).

Litvinov and colleagues (2012) have demonstrated that extension and compression of fibrin clots are mediated by the reorganisation of fibrin secondary structure, with α-helical segments converted into β-sheets (Zhmurov et al., 2012; Fleissner et al., 2016). Intriguingly, the α-helix to β-sheet transition was also associated with the formation of intermolecular β-sheet structures reminiscent of amyloid. This altered fibrin structure may affect clot lysis. Amyloid fibrils have a characteristic cross-β structure – where β-sheets align parallel to the fibril axis and individual β-strands extend perpendicularly to the axis (Chiti and Dobson, 2006; Eisenberg and Jucker, 2012). These fibrils are mechanically very stable due to hydrogen-bonding in the peptide backbone, as well as side-chain interactions (Smith et al., 2006; Knowles et al., 2007). Amyloid fibrils also exhibit a high degree of insolubility and resistance to proteolytic degradation. Moreover, it has been reported that fibrin clots from individuals living with chronic inflammatory conditions have a higher β-sheet content, compared to clots from healthy individuals, and that these aberrant fibrin networks are amyloid in nature (Kell and Pretorius, 2017; Pretorius et al., 2017; Pretorius et al., 2018a; Pretorius et al., 2018b).

In this chapter, potential differences in the secondary structure of fibrin clots from patients with PsO and healthy individuals were investigated. Fluorescence microscopy was used to determine the presence of areas that are rich in β-sheet structures (resembling amyloid), in fibrin clots. In addition, protein secondary structure content was quantitatively evaluated through Fourier transform infrared (FTIR) spectroscopy.

4.2 Materials and Methods

4.2.1 Ethical Clearance and Informed Consent

Please refer to **Chapter 3**, section **3.2.1**.

4.2.2 Study Design and Study Population

Please refer to **Chapter 3**, section **3.2.2**.

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4.2.3 Collection of Whole Blood and Preparation of Platelet-Poor Plasma

Please refer to **Chapter 3**, section **3.2.3**.

4.2.4 Preparation of Platelet-Poor Plasma Clots

Platelet-poor plasma (PPP) of patients with PsO and healthy control subjects was thawed from -80°C to room temperature. Plasma reaction mixtures, with a final volume of 300 µL each, were prepared by adding 30 µL $CaCl_2$ (7003, Haemonetics®, Boston, MA, USA) (final concentration 20 mM) and 15 µL thrombin (provided by the South African National Blood Service, final concentration 1 IU/mL) to 255 uL PPP. Subsequently, reaction mixtures were transferred to 3 mL syringes and incubated at 37°C for 2 h. Afterwards, clots were carefully removed from the syringes and washed five times for 5 min with 1X Gibco® phosphate-buffered saline (PBS), pH 7.4. Fixation was performed by covering the samples with 4% paraformaldehyde for 1 h. Samples were washed again five times for 5 min with PBS. Clots were transferred to labelled embedding cassettes and processed with a Leica HistoCore PEARL tissue processor (14 0493 80101, Leica Biosystems, Nussloch, Germany) using an overnight program (**Table 4.1**). The main steps in the protocol included dehydration by immersing samples in a series of increasing ethanol concentrations, clearing with xylene, and infiltration of the samples by paraffin wax. Finally, samples were embedded in molten paraffin wax at 65°C, using a Leica EG1150H Paraffin Embedding Station (14 0388 881101, Leica Biosystems), and placed on a Leica EG 1150C Cold Plate (14 0388 80101, Leica Biosystems) to solidify. Samples were stored at room temperature until further processing.

Table 4.1 Automated tissue processing protocol that was used to process clots.

Step	Reagent	Duration (h)	Temperature (°C)
1	Ethanol (70%)	1.5	45
2	Ethanol (70%)	1.5	45
3	Ethanol (90%)	1.5	45
4	Ethanol (95%)	1.5	45
5	Ethanol (95%)	1.5	45
6	Ethanol (100%)	1.5	45
7	Ethanol (100%)	1.5	45
8	Ethanol (100%)	1.5	45
9	Xylene	1	45
10	Xylene	1	45
11	Xylene	1	45
12	Paraffin	1.5	65
13	Paraffin	1.5	65
14	Paraffin	1.5	65

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4.2.5 Fluorescence Microscopy

In order to detect the presence of β-sheet structures in fibrin clots from healthy individuals and patients with PsO, Congo red staining was performed on the previously prepared sections. Congo red is a diazo dye used for the detection of amyloid. Staining with Congo red is currently regarded as the gold standard for amyloid diagnosis (Sipe et al., 2016). Amyloid stained with Congo red exhibits green, yellow, or orange birefringence when visualised under polarised light. However, it has been suggested that Congo red fluorescence may be a more sensitive and specific method for the detection of amyloid, especially if only small amounts are present (Sen and Başdemir, 2003; Giorgadze et al., 2004; Clement and Truong, 2014).

Samples were trimmed and sectioned at a thickness of 10 µm using a Leica RM2125 RTS rotary microtome (14 0457 80101, Leica Biosystems). Sections were floated on a heated water bath (45°C) (Kunz Instruments AB, Nynäshamn, Sweden) and picked up on microscope slides. The alkaline Congo red technique (Puchtler et al., 1962) was used to detect β-sheet secondary structures. The following stock solutions were prepared: saturated sodium chloride (NaCl) in 80% ethanol (stock solution A) and saturated Congo red (C2677, Sigma-Aldrich) in 80% ethanol saturated with NaCl (stock solution B).

Prior to staining, sections were incubated at 65°C for 10 min, deparaffinised with xylene, and hydrated by passing sections through a series of decreasing ethanol concentrations – two changes of 100% ethanol for 2 min each, 90% ethanol for 1 min, and 70% ethanol for 1 min – and water for 1 min. Afterwards, sections were stained with Mayer's haematoxylin (C.I. 75290, Sigma-Aldrich) for 15 min, and blueing was performed by immersing samples in Scott's tap water for 8 min. Thereafter, sections were placed in an alkaline NaCl solution that was prepared by adding 1 mL of 1% sodium hydroxide (NaOH) to 100 mL of stock solution A, followed by filtration. Subsequently, sections were placed directly into Congo red solution for 20 min. The solution was prepared by adding 1 mL of 1% NaOH to 100 mL of stock solution B, followed by filtration. Samples were dehydrated by three immersions into 100% ethanol, and cleared with xylene. Finally, slides were dried on a slide dryer and mounted with glass coverslips using DPX Mountant for histology (06522, Sigma-Aldrich). Samples were visualised with an Axio Observer 7 inverted microscope (Zeiss) equipped with a Colibri 7 LED illumination system (Zeiss), and a Plan-Apochromat 63x/1.40 Oil DIC M27 Objective (Zeiss). Congo red fluorescence was evaluated using an excitation filter of 592 nm and an emission filter of 614 nm. Three micrographs of representative areas were captured per sample, under both brightfield and fluorescence illumination, using ZEN microscopy software.

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4.2.6 Fourier Transform Infrared Spectroscopy

To ascertain if there were any differences in the secondary structural composition of fibrin clots from individuals with PsO, compared to clots from healthy control subjects, FTIR spectroscopy was performed. FTIR spectroscopy is a valuable method for the characterisation of the secondary structure of peptides and proteins (Kong and Yu, 2007). The technique is based on the vibration of the bonds between the atoms in a molecule, as it absorbs infrared radiation. FTIR spectra are generally presented as a plot of the intensity of the absorption of infrared radiation versus the wavenumber. Specific functional groups are associated with prominent absorption bands, and polypeptides and proteins give rise to nine distinctive bands (Kong and Yu, 2007). These bands have been designated amide A, amide B, and amide I through to VII. The amide I absorption band (1600–1700 cm^{-1}) originates primarily from the C=O stretching vibration of the amide group, as well as smaller contributions from the C–N stretching and N–H bending modes (Jackson and Mantsch, 1995; Barth, 2007; Kong and Yu, 2007). The amide I band is the most useful in the determination of the secondary structural elements of proteins, as well as protein conformational changes (Kong and Yu, 2007).

Samples were trimmed and sectioned at a thickness of 10 µm using a Leica RM2125 RTS rotary microtome (14 0457 80101, Leica Biosystems). Sections were floated on a heated water bath (45°C) (Kunz Instruments AB) and picked up on microscope slides. Slides were covered with aluminium foil to compensate for the strong infrared absorption of glass (Cui et al., 2016). Sections were incubated at 65°C for 10 min, deparaffinised with xylene, and hydrated by passing the sections through a series of decreasing ethanol concentrations – two changes of 100% ethanol for 2 min each, 90% ethanol for 1 min, and 70% ethanol for 1 min – and water for 1 min. Finally, the slides were left to air-dry overnight.

Spectral measurements were acquired at room temperature using a Bruker Alpha P-ATR spectrometer (Bruker Optics Inc., Billerica, MA, USA) equipped with a platinum diamond cell attenuated total reflectance module. A background spectrum was recorded before the analysis of each sample was performed. Spectra were recorded in the spectral region of 500–4000 cm^{-1} at a resolution of 4 cm^{-1}. An example of an FTIR spectrum is provided in **Figure 4.1**. For each spectrum, 128 scans were averaged. Atmospheric compensation, to minimise interference from atmospheric water vapor and carbon dioxide, was performed using Bruker OPUS Spectroscopy version 8.5 software. Spectral correction was performed using a rubber-band baseline correction.

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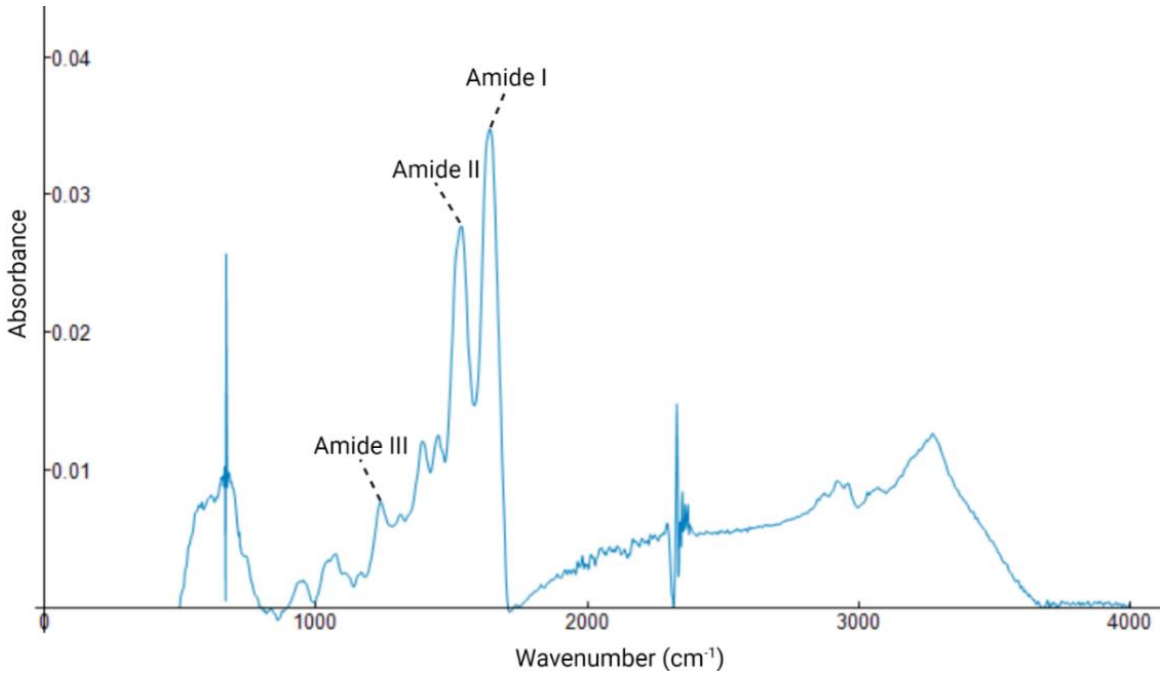

Figure 4.1 A representative Fourier transform infrared spectrum of a fibrin clot from a healthy individual. The graph was produced with Fityk version 1.3.1 software.

The amide I band comprises several overlapping bands and second-derivative analysis was applied to resolve individual band components (**Figure 4.2**). Second-derivative spectra were calculated and smoothed using an 11-point Savitzky-Golay smoothing algorithm in OriginPro® version 2021b software. Curve-fitting was performed using the non-linear least-squares method, assuming a Gaussian profile, in Fityk version 1.3.1 software (Wojdyr, 2010). To determine the relative contribution of different secondary structural elements to the clot structure, the area under the curve was calculated for each band and expressed as a percentage of the sum of the areas of all component bands of the amide I region.

4.2.7 Statistical Analysis

GraphPad Prism version 8.4.3 software was used to perform statistical analyses. Data were tested for normality using the Shapiro–Wilk normality test. To determine differences between groups, Student's t-tests were used for normally distributed data, while Mann–Whitney *U* tests were used for non-normally distributed data. Data are presented as the mean and standard error of the mean. A *p*-value of less than 0.05 was considered statistically significant.

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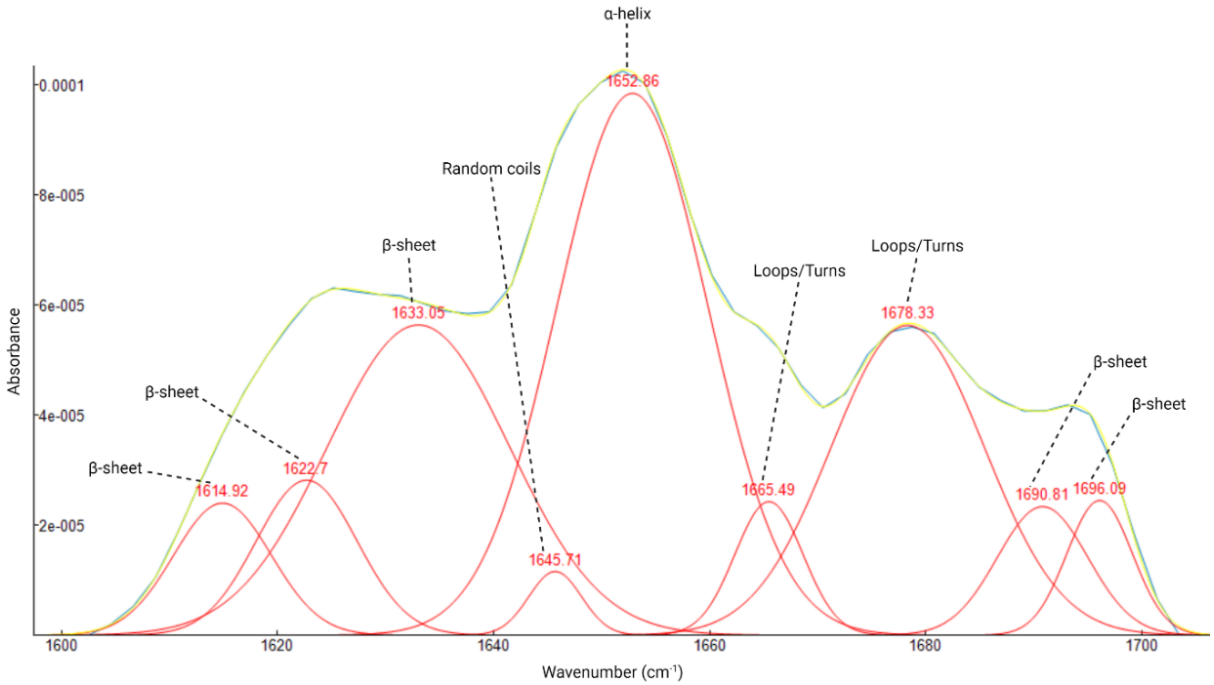

Figure 4.2 The curve-fitted (red functions) and inverted second-derivative spectrum (green function) of the amide I band of the original Fourier transform infrared spectrum shown in **Figure 4.1**. The graph was produced with Fityk version 1.3.1 software.

4.3 Results

The demographic features of healthy individuals and patients with PsO are presented in **Table 4.2**. There were no significant differences in age (p=0.126) and gender (p=0.702) between the study groups.

Table 4.2 Demographic features of healthy individuals and patients with psoriasis. Data are expressed as mean ± standard error of the mean.

Demographics	**Healthy individuals (n=10)**	**Patients with PsO (n=20)**
Age, (years)	60.600 ± 3.751	54.250 ± 2.148
Gender Female, n (%) Male, n (%)	 3 (30) 7 (70)	 8 (40) 12 (60)

Abbreviations: PsO, psoriasis.

A higher β-sheet content may influence the mechanical properties of a clot, as well as clot dissolution. A Congo red staining procedure was applied to detect areas rich in β-sheet structures in fibrin clots from individuals with PsO, compared to clots from healthy individuals. Representative micrographs from healthy control subjects and individuals with PsO are presented in **Figure 4.3** and **Figure 4.4**, respectively. Under brightfield microscopy, fibrin fibres had a deep pink-purple hue, while areas that stained positive for Congo red displayed an orange-red colour. Upon examination with fluorescence microscopy, bright red fluorescence was seen in areas that were Congo red positive. These areas also correlated with each other, with respect to location and appearance, under brightfield and fluorescence illumination.

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Compared to areas that exhibited Congo red fluorescence, the fibrin network presented with a more subdued fluorescent signal. As shown in **Figure 4.3A2–C2** and **Figure 4.4A2–C2**, Congo red fluorescence was detected in fibrin clots from both the control subjects and individuals with PsO. These areas seemed to be smaller and more dispersed in healthy individuals, with a weaker fluorescent signal (**Figure 4.3A2–C2**), whereas more prominent areas were detected in fibrin clots from patients with PsO (**Figure 4.4A2** and **C2**). In general, areas positive for Congo red appeared granular (**Figure 4.4A1**) or were localised to fibre branch points of the fibrin network.

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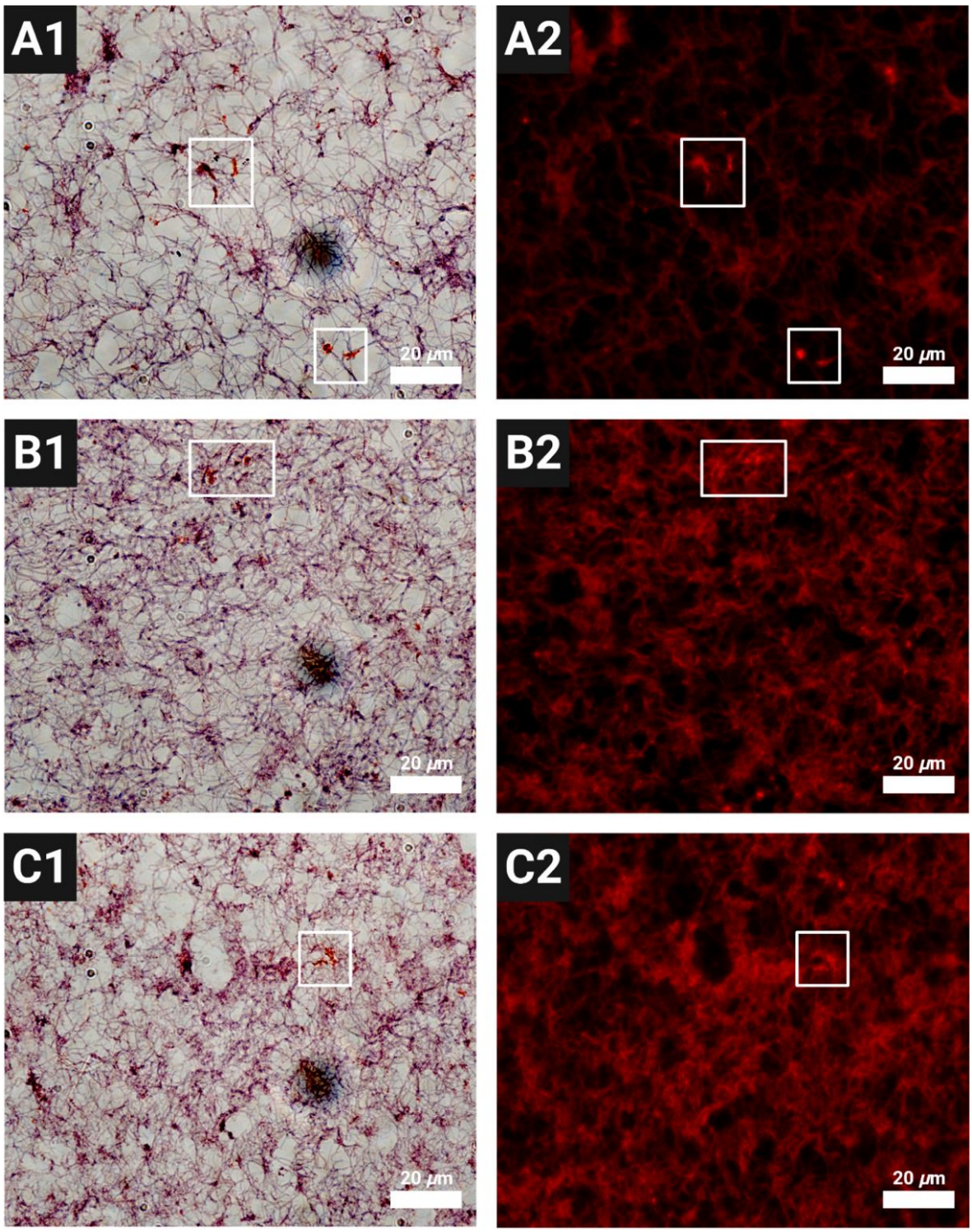

Figure 4.3 Representative micrographs of fibrin clots from healthy individuals. (**A1**), (**B1**), and (**C1**) show brightfield images. (**A2**), (**B2**), and (**C2**) show fluorescence images. Congo red fluorescence is indicated by the white boxes.

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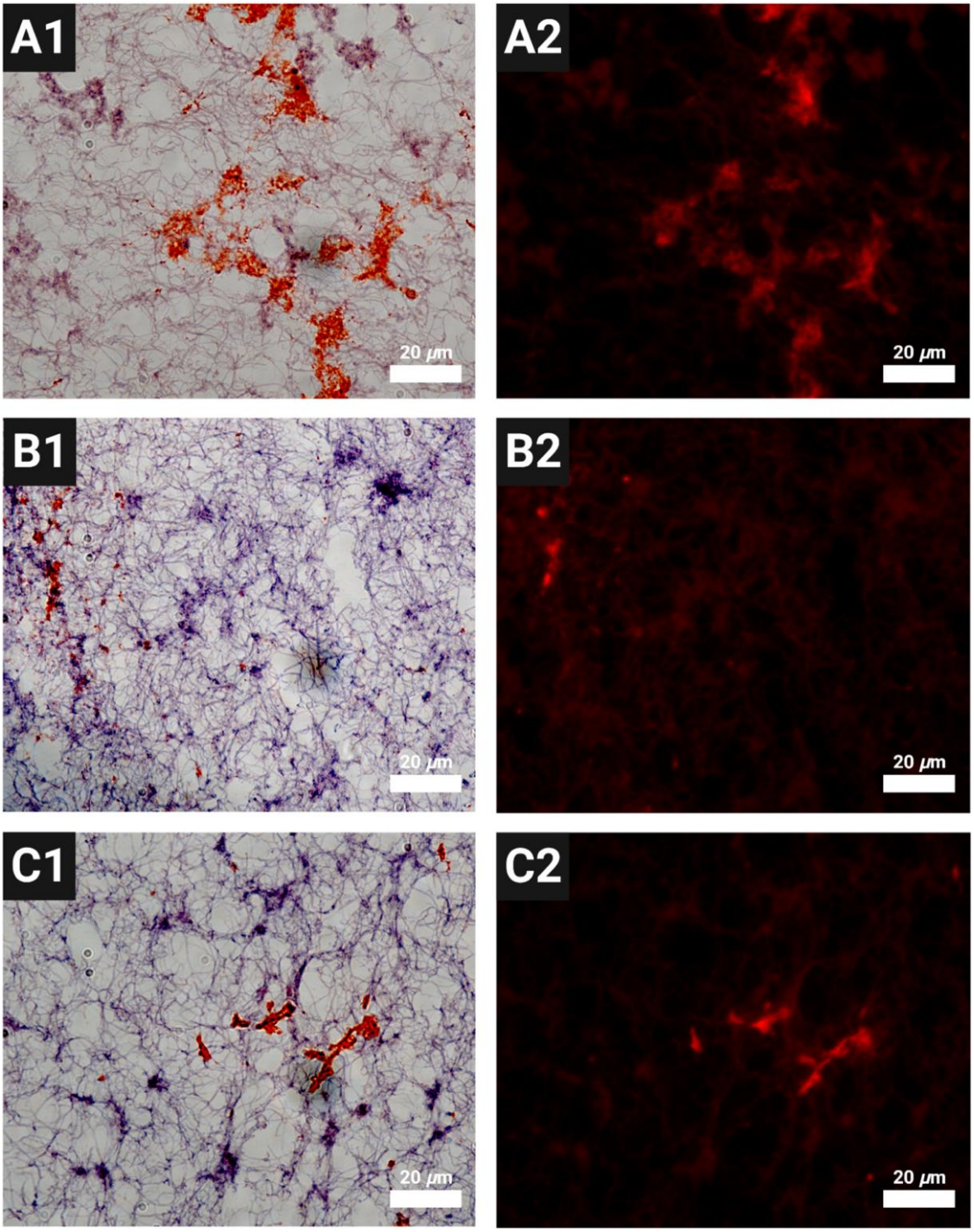

Figure 4.4 Representative micrographs of fibrin clots from patients with psoriasis. (**A1**), (**B1**), and (**C1**) show brightfield images. (**A2**), (**B2**), and (**C2**) show fluorescence images.

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FTIR spectroscopy was performed to determine if there were any differences in the secondary structure content of fibrin clots from patients with PsO, compared to those from healthy control subjects. The average FTIR spectra of fibrin clots from healthy individuals and individuals with PsO are shown in **Figure 4.5A** and **B**, respectively. Fibrin gave rise to three characteristic absorption bands at 1646 ± 1 cm^{-1}, 1536 cm^{-1}, and 1239 ± 2 cm^{-1}. First, the peak at 1646 ± 1 cm^{-1} is associated with the amide I band and originates from the C=O stretching vibration. Second, the peak at 1536 cm^{-1} corresponds to the amide II band and can be attributed to the N–H bending and C–N stretching vibrations. Third, the peak at 1239 ± 2 cm^{-1} represents the amide III band and arise from the N–H bending and C=O bending vibrations.

A quantitative estimation of the secondary structure content of fibrin clots was performed by calculating the second-derivative spectrum of the amide I region and, subsequently, applying a curve-fitting procedure (**Figure 4.5C** and **D**). Deconvolution of the amide I region revealed nine component bands. Band assignment (shown in **Table 4.3**) was based on experimental results of previously published studies (Dong et al., 1990; Venyaminov and Kalnin, 1990; Bramanti et al., 1997; Litvinov et al., 2012). The relative contribution of each type of secondary structural element to fibrin clot structure, in healthy individuals and patients with PsO, is presented in **Table 4.4**.

Table 4.3 Deconvoluted amide I band frequencies and secondary structure assignments.

Mean peak frequency (cm^{-1})		**Band assignment**
Healthy individuals	**Patients with PsO**	
1614.647	1615.314	β-sheet
1622.194	1622.868	β-sheet
1631.329	1630.969	β-sheet
1644.931	1646.087	Random coil
1652.559	1652.645	α-helix
1665.718	1666.364	Loop/Turn
1679.367	1680.096	Loop/Turn
1691.792	1691.661	β-sheet
1696.890	1696.722	β-sheet

Abbreviations: PsO, psoriasis.

Table 4.4 Percentage contribution of specific secondary structural elements to fibrin clot structure in healthy individuals and patients with psoriasis. Data are expressed as mean ± standard error of the mean.

Secondary structural element	**Healthy individuals (n=10)**	**Patients with PsO (n=20)**	***p*-value**
α-helix (%)	34.920 ± 1.995	35.740 ± 1.206	0.914
β-sheet (%)	40.190 ± 0.909	41.570 ± 0.769	0.475
Coils, loops, turns (%)	24.890 ± 1.947	22.440 ± 1.122	0.198

Abbreviations: PsO, psoriasis.

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Fibrin clots from healthy individuals contained 35 ± 2% α-helices, 40 ± 1% β-sheets, and 25 ± 2% random coils, loops, and turns (**Table 4.4**). Similarly, fibrin clots from patients with PsO consisted of 36 ± 1% α-helices, 42 ± 1% β-sheets, and 22 ± 1% random coils, loops, and turns. No significant differences were observed when the percentage values of the specific secondary structural elements in fibrin clots from patients with PsO were compared to those from control subjects.

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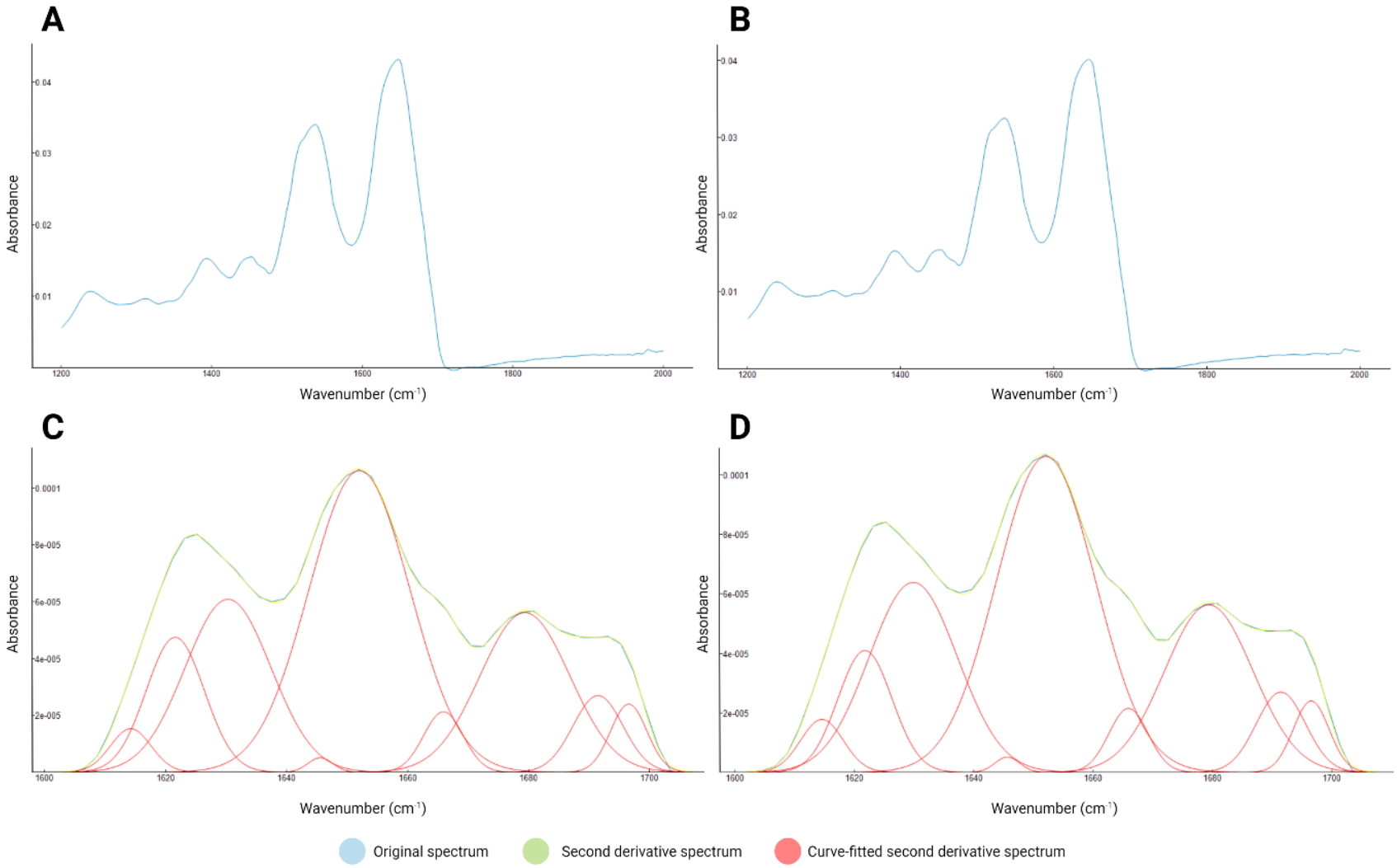

Figure 4.5 Average Fourier transform infrared (FTIR) and curve-fitted second-derivative spectra of fibrin clots from healthy individuals and patients with psoriasis (PsO). (**A**) and (**B**) Average FTIR spectra (in the 1200–2000 cm^{-1} region) of fibrin clots from healthy individuals and patients with PsO, respectively. (**C)** and (**D**) Curve-fitted inverted second-derivative spectra of the amide I region of fibrin clots from healthy individuals and patients with PsO, respectively. Graphs were produced with Fityk version 1.3.1 software.

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4.4 Discussion

Mounting evidence strengthens the notion that PsO is not limited to cutaneous symptoms and should rather be viewed as a systemic inflammatory condition (Grozdev et al., 2014; Korman, 2020). Similar to other chronic inflammatory conditions, the presence of psoriatic disease confers a significantly increased risk of cardiovascular events (Arnett et al., 2019). The exact mechanisms that underpin this association are yet to be elucidated. However, a myriad of dysregulated inflammatory molecules in PsO may drive a prothrombotic state in individuals with this condition. In **Chapter 3**, it was shown that a hypercoagulable state is indeed present in patients with PsO. This altered haemostatic profile was also accompanied by changes in the fibrin network architecture. A prothrombotic fibrin clot phenotype has been associated with venous and arterial thrombosis. In addition, clot mechanical properties, which is governed by the hierarchical structure of the fibrin network (Piechocka et al., 2010; Litvinov and Weisel, 2017), may also influence the outcome of an occlusive thrombus. It has been shown that an increase in clot stiffness is accompanied by an α-helix to β-sheet transition, as well as the formation of intermolecular β-sheets (Litvinov et al., 2012; Fleissner et al., 2016). Furthermore, it has also been proposed that fibrin may adopt an amyloid form under inflammatory conditions (Kell and Pretorius, 2017; Kell and Pretorius, 2018). In this study, no significant differences were found in the distribution of secondary structure elements in fibrin clots from patients with PsO, when compared to clots from healthy individuals. An illustrative summary of this study is provided in **Figure 4.6**.

In order to detect the presence of β-sheet-rich areas in sections of fibrin clots, the alkaline Congo red staining method was used. Fluorescence microscopy revealed the presence of congophilic material in fibrin clots from both patients with PsO and control subjects. However, there were no apparent differences in the abundance of Congo red-positive areas between clots from individuals with PsO and those from healthy individuals. A possible explanation could be that the observed fluorescence signal was caused by non-specific staining of fibrinogen. However, the staining protocol that was used here has been modified to increase the sensitivity of Congo red. Indeed, in retrospective studies of samples with known amyloid deposition, Congo red fluorescence readily confirmed the diagnosis (Sen and Başdemir, 2003; Giorgadze et al., 2004; Marcus et al., 2012; Clement and Truong, 2014). Moreover, others have reported that fibrin clots from individuals with chronic inflammatory diseases are amyloid in nature (Pretorius et al., 2017; Pretorius et al., 2018a; Pretorius et al., 2018b). It has also been demonstrated, in a series of studies by Strickland and colleagues, that fibrinogen may interact with β-amyloid peptide, resulting in the generation of aberrant fibrin clots that are resistant to plasmin-mediated fibrinolysis (Ahn et al., 2010; Zamolodchikov and Strickland,

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2012; Cajamarca et al., 2020). These findings suggest that fibrinogen might have some amyloidogenic potential.

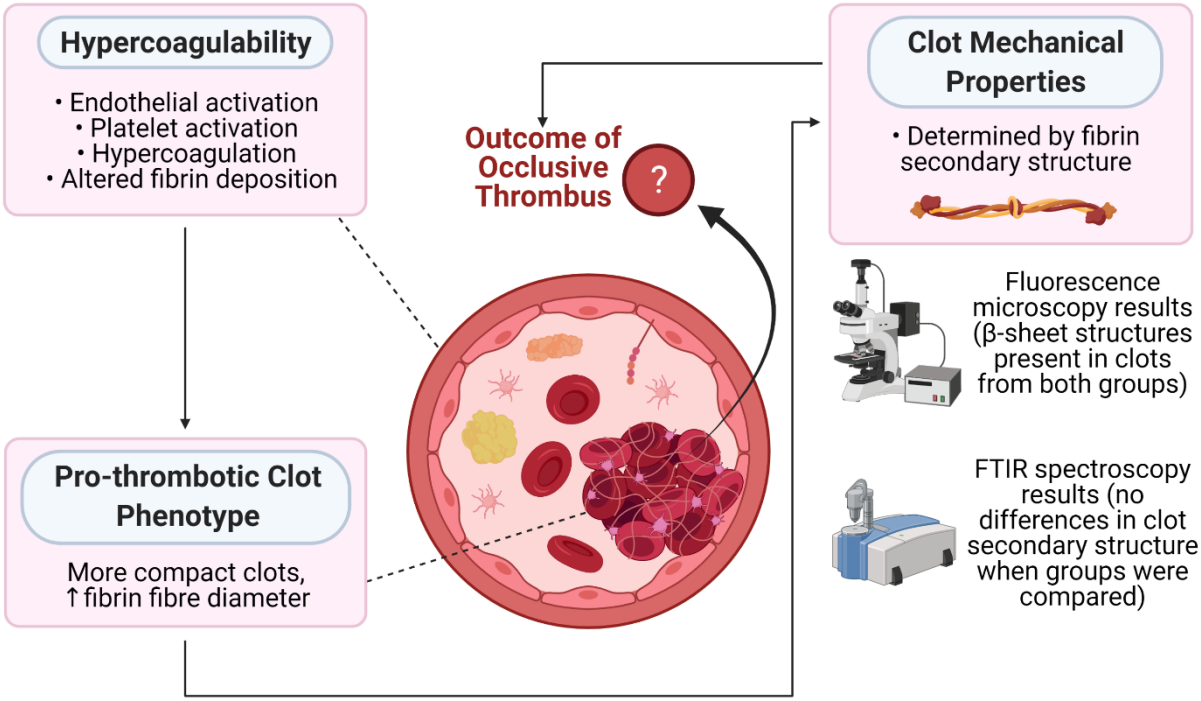

Figure 4.6 A summary of this study. Chronic systemic inflammation, due to psoriatic pathology, may drive the development of a prothrombotic state in individuals living with psoriasis (PsO). An altered fibrin clot structure has been associated with an increased thrombotic tendency. The fibrin network structure, at various levels, determines clot mechanical properties. To a large extent, clot mechanics dictate the potential outcome(s) of an obstructive thrombus. In this study, the secondary structure content of fibrin clots from patients with PsO and healthy individuals was investigated using fluorescence microscopy and Fourier transform infrared spectroscopy. No meaningful differences were found when the secondary structure composition of fibrin clots from patients with PsO were compared to those of healthy controls. Diagram created with BioRender.com. **Abbreviations:** FTIR, Fourier transform infrared.

In view of the above, FTIR spectroscopy was performed on sections of fibrin clots from patients with PsO and healthy individuals to quantitatively determine the contribution of specific secondary structural elements. After spectral analysis, and based on the band assignments in **Table 4.3**, it was found that there were no significant differences (**Table 4.4**) in the amounts present of the different secondary structural elements in fibrin clots between the groups. These results are consistent with previous FTIR (Azpiazu and Chapman, 1992; Bramanti et al., 1997; Litvinov et al., 2012) and Raman (Marx et al., 1979; Fleissner et al., 2016) spectroscopy studies that quantified the secondary structure of native human fibrin. In contrast to the findings of this study, it has been reported that fibrin(ogen) assumes an amyloid-like form in a pro-inflammatory milieu (Pretorius et al., 2017; Pretorius et al., 2018a; Pretorius et al., 2018b). A possible explanation for this discrepancy may be the different techniques that were used to assess fibrin secondary structure. In the aforementioned studies, only fluorescence microscopy was utilised to evaluate potential amyloidogenic changes in fibrin clots, whereas a

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qualitative staining method in combination with a quantitative FTIR spectroscopy analysis were used in this study.

Taken together, it can be concluded that the secondary structure of fibrin is not altered in patients with PsO, compared to fibrin clots from healthy controls. Therefore, it may also be inferred that the mechanical properties of clots from the two groups were similar. This is reflected by the TEG results, presented in **Chapter 3**, which indicated that clot stiffness was comparable between groups. Regarding the presence of β-sheet structures in clots, it should be considered that fibrin may inherently contain small quantities of misfolded and/or unfolded protein. It has recently been shown that unfolded fibrinogen is incorporated into fibrin clots, and that cross-β-sheet structures are generated during coagulation (Talens et al., 2019). This would explain the presence of β-sheet structures, as detected by fluorescence microscopy, in fibrin clots from both groups, as well as the absence of significant differences in fibrin secondary structure composition, as determined by FTIR analysis, between the groups. Finally, it can also be speculated that the tertiary structure, rather than the secondary structure, of fibrin was disrupted in the present study. It has been demonstrated that the addition of reactive carbonyl compounds to fibrinogen gave rise to aggregates with amyloid-like properties, although fibrinogen secondary structure remained unaltered (Xu et al., 2012). It was suggested that these aggregates were molten globules – a protein conformational state where secondary structure is native-like, but tertiary structure is highly disordered.

This study has a number of limitations. First, supplementary methods, such as immunohistochemistry, were not used to confirm that the areas that exhibited Congo red fluorescence were indeed amyloid. Second, PPP clots were assessed in this study, although *in vivo* clots contain other additional blood components, such as red blood cells and platelets, that may impact clot mechanical properties (Tutwiler et al., 2016).

4.5 Conclusion

Individuals living with PsO have been shown to be at an increased risk of cardiovascular complications, and hypercoagulability – driven by chronic systemic inflammation – may be an important contributing factor to this relationship. In addition to thrombotic propensity and fibrin structure, the mechanical properties of a clot may also influence thrombosis outcomes. In response to deformation, fibrin clots may undergo an α-helix to β-sheet transition and form structures rich in β-sheets – akin to amyloid. It has been proposed that fibrin may assume an amyloid form in a pro-inflammatory milieu. Here, the secondary structure composition of fibrin clots from patients with PsO and healthy controls, was characterised. No statistically significant differences were found when clot structure was compared between the groups. However, it

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should be recognised that the current *in vitro* model has limitations and does not fully recapitulate the pathophysiological process of thrombosis.

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Chapter 5: General Conclusion

Psoriasis (PsO) has been recognised as a serious non-communicable disease. The condition has been associated with a variety of comorbidities, including cardiovascular disease (CVD). A potential pathophysiological mechanism that may contribute to the increased prevalence of cardiovascular events, among patients with PsO, is the presence of a hypercoagulable state. Accordingly, the aim of this study was to assess the haemostatic profile of patients with PsO, in relation to healthy control subjects. In addition, potential alterations in fibrin clot structure in patients with PsO were also investigated. The results of this study indicate that a hypercoagulable state exists in individuals living with PsO.

Elevated plasma levels of acute-phase reactants, C-reactive protein (CRP) and serum amyloid A, were associated with disease status, thereby confirming the presence of systemic inflammation in patients with PsO (**Chapter 3**; Visser et al, 2021). In addition, endothelial and platelet activation – reflected by increased soluble intercellular adhesion molecule-1 and soluble P-selectin levels – were also related to disease presence. Changes in whole blood coagulation parameters, as assessed by thromboelastography (TEG), were associated with the presence of PsO, and suggested an increased tendency towards clot formation in patients with PsO. However, contrary to expectation, clots from patients with PsO did not exhibit an increase in clot stiffness, when compared to control subjects. This unexpected result could be attributable to the effects of immunosuppressive drugs (Paul et al., 2015), or platelet refractoriness due to hyperactivation (Tutwiler et al., 2017). Impaired clot contraction, potentially due to an inadequate platelet response, may heighten the risk of embolisation. For the first time, it was demonstrated that fibrin clot ultrastructure is altered in patients with PsO. Clots from these patients were more compact and composed of thicker fibrin fibres, relative to clots from healthy controls. An increased fibrin fibre diameter also correlated with disease presence. Regarding the first part of this study, future research could include investigation of the levels of inflammatory cytokines, namely interleukin (IL)-1β, IL-6, IL-17, and tumour necrosis factor (identified in **Chapter 2**), in patients with PsO and potential correlation(s) with disease severity and/or TEG clotting parameters. In particular, the CRP/IL-6/IL-1 axis has been recognised as a central signalling pathway in the pathogenesis of CVD (Ridker, 2016). Furthermore, fibrinolytic activity could be assessed, as hypofibrinolysis has been associated with arterial and venous thrombotic events (Meltzer et al., 2010; Kietsiriroje et al., 2021).

As discussed in **Chapter 4**, additional analyses were performed to characterise clot structure at the molecular level using a combined qualitative and quantitative approach. No significant differences were found in the secondary structural composition of fibrin clots from patients with PsO, compared to those from healthy individuals. Areas that were rich in β-sheet structures were detected in fibrin clots from both groups. Furthermore, quantitative Fourier transform

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infrared analysis did not reveal any significant differences with regard to the amounts present of specific secondary structures in clots from patients with PsO and controls. These findings are in agreement with TEG results that indicated that clot stiffness did not differ between groups. If clot strength was increased, one would have expected a greater contribution of β-sheets to fibrin secondary structure. Future research could include immunohistochemical staining for 'amyloid signature proteins', such as serum amyloid P component, that co-deposit with amyloid, as a complementary method to Congo red fluorescence to confirm the presence of β-sheet structures (Benson et al., 2020). Subsequent research could also be carried out on thrombi that were retrieved from patients with PsO in response to adverse thrombotic events. These clots would be more reflective of the pathophysiological environment *in vivo*, and also incorporate the contributions of other cellular components and biophysical forces to clot mechanical properties. Alternatively, fibrin clots prepared from the plasma of individuals with PsO with confirmed thrombosis and those without thrombosis, could also be compared. The question as to whether fibrin assumes an amyloid form under inflammatory conditions still remains to be answered definitively. Based on the current literature, it seems that mechanical deformation is required to induce the structural rearrangement of fibrin, and consequently, alter the mechanical behaviour of the fibrin network. However, it would appear that the secondary structure of fibrin may also be altered in response to specific post-translational modifications [i.e., glycation (Perween et al., 2019) and carbonylation (Becatti et al., 2014; Becatti et al., 2016)] and oxidative stress (Wang et al., 2016). Future studies of changes in the molecular structure of fibrin might be particularly relevant, as these changes may also impact the biochemical properties of fibrin. A recent study by Kumar and colleagues (2022) has demonstrated that the α-helix to β-sheet transition resulted in reduced binding of tissue-type plasminogen activator, as well as modulation of the platelet response.

This study had limitations. First, as this was an observational cross-sectional study, causality cannot be attributed. Longitudinal studies would have to be conducted to demonstrate any causal relationships; however, observational studies are important to study initial associations. Second, the patient population in this study was heterogenous, as patients suffered from a range of comorbidities and were also treated with a variety of therapies, ranging from topical corticosteroids to biological agents. It should be noted that it is challenging to identify patients with PsO that are treatment-naïve, due to the disfiguring nature of the condition. Furthermore, PsO is a complex condition and due to its systemic effects, one can assume that most patients living with PsO will present with a high burden of comorbidities. In this study, the potential confounding effects of comorbid conditions may have been addressed more effectively by allowing for the presence of certain conditions in the control group, to ensure an equal distribution of comorbidities across the two groups.

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Regarding future studies of the association between PsO and CVD, there are various questions that warrant further investigation. Immunothrombosis – thrombosis linked to the innate immune response (Engelmann and Massberg, 2013) – might be a particularly relevant topic to study in the context of PsO. Briefly, neutrophil extracellular traps (NETs) are formed when the nuclear constituents and granular proteins are released by neutrophils in response to pathogens (Brinkmann et al., 2004). NET formation has been implicated in PsO pathogenesis in recent years (Lin et al., 2011; Shao et al., 2019; Herster et al., 2020). NETs may act as a scaffold for the adhesion and aggregation of platelets and may also recruit red blood cells (Fuchs et al., 2010). These networks may also be integrated into fibrin clots, promoting clot stability (Fuchs et al., 2010). Furthermore, as pointed out in a recent review by Aksentijevich and colleagues (2020), PsO presents a unique opportunity as a human model for the study of chronic systemic inflammation in the development of CVD. Treatment of patients with PsO with targeted biological therapies may shed light on the contribution of specific cytokines to cardiovascular morbidity, and provide novel treatment targets.

PsO is a common immune-mediated inflammatory disease of multifactorial aetiology, that confers an increased risk of CVD. In this dissertation, a mechanism that may link PsO to CVD risk, namely an acquired hypercoagulability, has been explored. Patients with PsO have an altered haemostatic profile, characterised by an increased propensity for clot formation. In addition, it was demonstrated for the first time that fibrin clot ultrastructure is altered in patients with PsO. Understanding the association between PsO and CVD at a pathogenic level is of importance, as this will not only influence the way in which PsO is managed, but also how CVD risk is addressed in these individuals.

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Appendix A: Ethical Approval Letter

UNIVERSITEIT
STELLENBOSCH
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Approval Letter
Amendment

17/06/2020

Project ID: 9521

Ethics Reference No: N19/03/043

Project Title: Circulating inflammatory biomarkers and dysregulated coagulation in inflammatory conditions.

Dear Prof Etheresia Pretorius

We refer to your amendment request received 12/06/2020.

The Health Research Ethics Committee (HREC) reviewed and **approved** the amendment through an expedited review process.

The following amended documentation was reviewed and **approved:**

- Protocol version 1, dated 2020_06_12

Where to submit any documentation

Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.

Please remember to use your project ID 9521 and ethics reference number N19/03/043 on any documents or correspondence with the HREC concerning your research protocol.

Yours sincerely,

Mrs. Melody Shana
Coordinator: Health Research Ethics Committee 1

National Health Research Ethics Council (NHREC) Registration Number:

REC-130408-012 (HREC1)•REC-230208-010 (HREC2)

Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)•IRB0005239 (HREC2)

The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by the
World Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects;the South African *Department of Health (2006). Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition)*; as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes and Structures (2nd edition).

The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46); and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.

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Appendix B: Participant Information Leaflet and Consent Form

UNIQUE ETHICS NUMBER: 9521 Left-over blood will be stored and used for similar studies, e.g., clearance numbers: 6399; 1952; 6983; 6592	**Control (mark with X)**
	Inflammatory disease (mark with X)
	NAME OF CONDITION_____

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT: Circulating inflammatory biomarkers and dysregulated coagulation in inflammatory conditions

REFERENCE NUMBER: 9521

PRINCIPAL INVESTIGATOR: Prof. E. Pretorius

ADDRESS: Department of Physiological Sciences, Faculty of Science, Stellenbosch University, STELLENBOSCH

CONTACT NUMBER: 0829295041

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary,** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice, and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

You are invited to participate in research study conducted by Prof Resia Pretorius. This information leaflet is to help you to decide if you would like to participate. Before you agree to take part, you should fully understand what is being involved in this research study. Your medical practitioner and/or phlebotomist will explain the reason for the drawing of blood. As part of this current research study, 4 blood tubes will be drawn from you. First tube will be sent to pathology laboratory or analyzed by ourselves, to determine your inflammatory status by looking at inflammatory markers like CRP levels. (An increase in CRP levels may influence the shape of the red blood cells, platelets, and fibrin). The other 3 tubes will be used for our laboratory for haematological (blood) analysis, using specialized microscopes and specialized equipment for biochemical blood molecule (biomarker) analyses.

The research team members are investigating the physiology and functioning of cells and circulating inflammatory molecules in your blood. This will be done by using specialized microscopes (that can magnify up to 100 000x (called an electron or a confocal microscope) and an instrument called a flow cytometer). We will also look at your blood using nanobiosensors, developed by prof Willie Perold and his research students from the Engineering Department. We

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will store a part of your sample that is not used, for later analysis in similar studies, so that we do not waste samples.

Why have you been invited to participate?
We are recruiting either healthy controls or individuals with inflammation and/or cardiovascular disease.
OR
Your medical practitioner wishes to obtain an individualized biomarker analysis of your inflammatory profile. He/she will discuss the results with you.

What will your responsibilities be?
You will donate 4 tubes of blood (20 ml). If you choose not to participate in this research study, you will still receive the standard care form your medical practitioner. You have no obligation to participate.

Will you benefit from taking part in this research?
There are no personal benefits, but results generated from this study will allow researchers to determine the physiology of healthy clotting and red blood cell structure and will be used to test novel nanobiosensors for blood clotting analysis, constructed by the members of the Faculty of Engineering, Stellenbosch University. If your medical practitioner has requested the analysis, via our SUNguis blood laboratory, he/she will share the results with you. We as SUNguis blood laboratory will not be involved in any diagnoses. We will simply provide your medical practitioner with results generated in our laboratory using our point-of-care blood analysis devices or state-of-the-art research equipment.

Are there risks involved in your taking part in this research?
The venipuncture (i.e., drawing blood) is normally done as part of routine medical care and presents a slight risk and discomfort. Drawing blood may result in a bruise at the puncture site, or less commonly swelling of the vein, infection, and bleeding from the site. For your protection, the procedures will be performed under sterile conditions by your medical practitioner or by a trained phlebotomist.

If you do not agree to take part, what alternatives do you have?
None

Who will have access to your medical records?
There will be access to any medical records only to determine previously obtained glucose levels and cholesterol levels. ***All information obtained during the course of this study is strictly confidential.*** *Data that may be reported in scientific journals will not include any information, which identifies you as a patient in this study. We will ONLY have your name on this informed consent form but will give your blood tubes a dedicated UNIQUE number so that you will not be identified in any way. Prof Pretorius and the team will only use the unique number in the data analysis. Samples that are not fully used, will be stored under the unique number for future (repeat or similar) tests.*

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?
Neither you nor your medical scheme will be expected to pay for the study. During a study-related injury, the Department of Physiological Sciences/ Prof Pretorius assume no obligation to pay for the medical treatment of other injuries. You may discuss this in detail with your medical practitioner or Pathology Laboratory who have insurance for injuries that might occur during routine blood drawing practices.

Will you be paid to take part in this study and are there any costs involved?
No, you will not be paid to take part in the research study.
If your medical practitioner requests the personalized biomarker and coagulation analysis, payment will be done via the SUNguis system.

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Is there anything else that you should know or do?

- You can contact the Health Research Ethics Committee at 021 938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- If you request it, you will receive a copy of this information and consent form for your own records.

Age:
Gender:
HbA1c levels:
Cholesterol levels:

Other medication used:

Declaration by participant

By signing below, I ……..……. agree to take part in a research study "**Circulating inflammatory biomarkers and dysregulated coagulation in inflammatory conditions**"
OR because my medical practitioner requested a personalized biomarker and/or coagulation analysis via the SUNguis incubator blood laboratory.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) .....……..…….. on (*date*) ……...……..

.....	.....
Signature of participant	**Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

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Signed at (*place*) .....…..…….. on (*date*) ……...……..

....
Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) …….. to explain the information in this document to (*name of participant*) ……..…….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…..…….. on (*date*) ……...……..

....
Signature of interpreter **Signature of witness**

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Appendix C: Publication

frontiers in Immunology

REVIEW
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Thrombosis in Psoriasis: Cutaneous Cytokine Production as a Potential Driving Force of Haemostatic Dysregulation and Subsequent Cardiovascular Risk

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Psoriasis (PsO) is a common T cell-mediated inflammatory disorder of the skin with an estimated prevalence of 2%. The condition manifests most commonly as erythematous plaques covered with scales. The aetiology of PsO is multifactorial and disease initiation involves interactions between environmental factors, susceptibility genes, and innate and adaptive immune responses. The underlying pathology is mainly driven by interleukin-17. In addition, various inflammatory mediators from specific T helper (T_H) cell subsets, namely T_H1, T_H17, and T_H22, are overexpressed in cutaneous lesions and may also be detected in the peripheral blood of psoriatic patients. Moreover, these individuals are also at greater risk, compared to the general population, of developing multiple comorbid conditions. Cardiovascular disease (CVD) has been recognised as a prominent comorbidity of PsO. A potential mechanism contributing to this association may be the presence of a hypercoagulable state in these individuals. Inflammation and coagulation are closely related. The presence of chronic, low-grade systemic inflammation may promote thrombosis – one of the major determinants of CVD. A pro-inflammatory milieu may induce the expression of tissue factor, augment platelet activity, and perturb the vascular endothelium. Altogether, these changes will result in a prothrombotic state. In this review, we describe the aetiology of PsO, as well as the pathophysiology of the condition. We also consider its relationship to CVD. Given the systemic inflammatory nature of PsO, we evaluate the potential contribution of prominent inflammatory mediators (implicated in PsO pathogenesis) to establishing a prothrombotic state in psoriatic patients.

Keywords: cardiovascular disease, endothelium, hypercoagulability, platelets, psoriasis, systemic inflammation

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INTRODUCTION

Psoriasis (PsO) is a chronic immune-mediated inflammatory disease of the skin, often associated with multiple comorbidities, affecting approximately 2% of the global population (1). Of those affected, 30% may develop an inflammatory arthritis (psoriatic arthritis). The reported prevalence of PsO in childhood may be up to 1.37% (2–4), while the estimated prevalence in adults ranges from 0.51% to 11.43% (3, 5, 6). Research suggests that disease development involves a complex interplay between genetic predisposition, environmental stimuli, and disordered innate and adaptive immune responses. Lesions may assume a variety of clinical forms (7), with plaque PsO being the most common disease variant. Typically, the condition manifests as well-circumscribed, erythematous papules and/or plaques covered with scales. In addition to its physical symptoms, PsO also imposes a significant psychosocial burden that may lead to anxiety, depression, and in severe cases, suicidality (8). The physical and psychological impact of PsO may significantly influence a patients' quality of life (9), as well as contribute to an increased risk of mortality in severe forms of the disease (10).

PsO is considered to be a T cell-mediated disease and the corresponding cytokine profile of psoriatic lesions indicates important roles for interferon (IFN)-α, interleukin (IL)-22, the IL-23/IL-17 axis, and tumour necrosis factor (TNF) in psoriatic pathology (11–13). In addition to localised cutaneous inflammation, these molecules have also been detected in the systemic circulation of psoriatic patients – increasing the risk of comorbidities (14–16). Considering these observations, a paradigm shift has occurred from viewing PsO as merely 'skin-deep' to viewing it as a systemic inflammatory condition that can affect various extracutaneous tissues (17). Cardiovascular disease (CVD) is a notable comorbidity in patients suffering from PsO. An increased risk of major cardiovascular events, as well as an increased CVD mortality in severe forms of the disease, has been reported in these individuals (18–22). The relationship between PsO and CVD is widely acknowledged, however, the mechanisms responsible remain uncertain.

Hypercoagulability is a potential mechanistic link accounting for the association between CVD and PsO. It is becoming increasingly clear that inflammation and coagulation are interrelated processes (23, 24). A hypercoagulable state may develop as a result of an imbalance in haemostatic and inflammatory activity mediated by pro-inflammatory cytokines. These molecules may promote the initiation of coagulation, inhibit endogenous anticoagulant systems, and impair fibrinolytic activity (24, 25). The vascular endothelium also plays a central role in clot formation, as it is located at the nexus of inflammation and coagulation. Under normal physiological conditions, the resting endothelium displays anticoagulant and anti-inflammatory effects (26, 27). This homeostasis is disrupted in inflammatory conditions as a result of pro-inflammatory cytokines perturbing the vessel wall, resulting in a transition to an activated state that favours coagulation (28).

This review will briefly discuss genetic and environmental risk factors associated with the development of PsO, as well as the pathogenesis of the condition. In addition, the literature describing the systemic inflammatory nature of PsO and its subsequent relationship to CVD will also be considered. Finally, the potential contribution of prominent inflammatory mediators in PsO, to promote a prothrombotic state, is also appraised.

THE AETIOLOGY OF PSORIASIS

Genetic Risk Factors

The important role of a genetic component in the molecular pathogenesis of PsO has been supported by various family-based studies (29), including twin studies. An increased disease concordance rate has been reported in monozygotic twins, compared to dizygotic twins (30–32). Despite this disease concordance, the incidence never reaches 100% – suggesting a role for environmental factors in addition to genetic susceptibility in PsO development. The current view is that the mode of inheritance for PsO is multifactorial. Classic genome-wide linkage analysis has identified at least nine different chromosomal regions, termed psoriasis susceptibility (*PSORS1–PSORS9*) loci, which have shown statistically significant associations with PsO (33). Multiple studies have validated *PSORS1* as the most important genomic region in PsO predisposition and that it may account for up to 35% of disease heritability (34). *PSORS1* is located on human chromosome 6p21.3 within the region of the major histocompatibility complex (MHC). *Human leukocyte antigen (HLA)-Cw*0602* has been accepted as the most likely *PSORS1* disease allele (35). The *HLA-C* gene encodes a class 1 MHC protein and participates in the priming of cluster of differentiation (CD) 8^+ T cell immune responses. More than 80 PsO susceptibility loci have been identified in genome-wide association studies (GWASs), conducted mainly on European and Asian populations (36). In 2007, in the first GWAS for PsO, two genes (*IL12B* and *IL23R*) were identified that were associated with PsO risk (37). Specific pathways implicated in PsO pathogenesis that have been identified through GWASs include interferon signalling, the IL-23 pathway, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) cascade and the regulation of T cell responses (38–42).

Environmental Risk Factors

Environmental triggers play a critical role in the onset and development of PsO in genetically predisposed individuals through the interaction with genes and the induction of epigenetic modifications (43). Various environmental exposures have been associated with the initiation and/or exacerbation of psoriatic lesions. Stressful life events are well known to contribute to the initiation and aggravation of PsO. A potential explanation for stress-induced PsO onset or flares may be dysregulation of hypothalamic–pituitary–adrenal axis activity (44, 45). Obesity is another significant risk factor for PsO and body mass index has been demonstrated to correlate with disease severity (46). Potential mediators of this association may include adipokines, such as leptin and resistin, which possess

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pro-inflammatory actions (47). Furthermore, epidemiological studies have suggested an association between smoking and the development of PsO, based on the increased incidence of PsO reported among current and former smokers compared to non-smokers (48–50). Smoking may induce PsO by augmenting the existing systemic oxidative stress, interacting with immune cells, and altering gene expression (51, 52). Bacterial infections, specifically by *Staphylococcus aureus* and *Streptococcus pyogenes*, are recognised triggers of PsO. β-haemolytic streptococci have been isolated more frequently from throat swabs from PsO patients, compared to controls (53), and enterotoxins from *Staphylococcus aureus* have been linked to more severe PsO (54). Dysbiosis of both the gut and skin microbiome has become a recurrent theme in psoriatic individuals (55). Gut dysbiosis, characterised by a decrease in microbes with anti-inflammatory and immunomodulatory properties (56, 57), seems to be prevalent in these individuals (58, 59). An altered skin microbiome has also been associated with the condition (60, 61).

PATHOGENESIS OF PSORIASIS

Environmental factors (e.g., stress, obesity, smoking, infection, and dysbiosis) or physical trauma (the Koebner phenomenon) may perturb keratinocytes, resulting in the release of self-deoxyribonucleic acid (DNA) or self-ribonucleic acid (RNA) (62, 63). In turn, these self-nucleic acids may form a complex with the endogenous antimicrobial peptide (AMP), cathelicidin/LL37 (63). DNA-LL37 complexes activate plasmacytoid dendritic cells (pDCs), *via* Toll-like receptor (TLR) 9 signalling, which secrete high levels of IFN-α (64). In a similar fashion, RNA-LL37 complexes activate pDCs in a TLR7-dependent manner (62). Consequently, conventional dendritic cells (cDCs), which function as professional antigen-presenting cells (APCs), are activated by IFN-α. RNA-LL37 complexes also possess the capacity to activate cDCs directly, leading to the production of IL-6 and TNF by these cells. Once activated, cDCs migrate to secondary lymphoid organs and secrete IL-12 and IL-23 (65), which induce the differentiation of naïve $CD4^+$ T cells into T_H1 (66) and T_H17 (67) cells, respectively. This process provides a potential mechanism for the initiation of the inflammatory sequelae in PsO and highlights the central role of DCs to establish a link between the innate and adaptive branches of the immune system.

T_H1 cells express cytokines such as IFN-γ, IL-2, and TNF (66, 68). IFN-γ induces the expression of several chemokines and cytokines in the skin and also promotes the accumulation and infiltration of inflammatory cells (69, 70). TNF has been identified as a key regulatory molecule in the cytokine network of PsO. In addition to T_H1 cells, a distinct subset of DCs, namely TNF- and inducible nitric oxide synthase-producing DCs, also release large amounts of TNF (71). It has been suggested that TNF production mediates the proliferation of resident T cells in the development of psoriatic lesions (72). In addition, TNF regulates the expression of cell adhesion molecules, which mediate the extravasation of leukocytes, on endothelial cells and keratinocytes in psoriatic skin (73). TNF also stimulate keratinocytes to induce the expression of pro-inflammatory cytokines such as IL-6, IL-8, and TNF itself, through the activation of NF-κB (74). Furthermore, TNF may synergise with IL-17 to enhance the expression of key inflammatory genes in keratinocytes (75).

T_H17 cytokines such as IL-17A, IL-17F, and IL-22 (76, 77) are key pathogenic effectors in PsO. Therefore, PsO has been regarded as a T_H17-mediated disease. However, this paradigm is shifting towards understanding PsO as an IL-17-driven disease (78). Various other cellular sources of IL-17 exist, including $CD8^+$ T cells (comprising mucosa-associated invariant T cells and conventional T cells) (79), dermal γδ T cells (80), group 3 innate lymphoid cells (81), mast cells, and neutrophils (82). IL-17A has been shown to govern the expression of signature PsO genes in keratinocytes (83). IL-17 acts on keratinocytes to induce the expression of AMPs, such as β-defensin 2, and neutrophil chemoattractants, namely C-X-C motif chemokine ligand (CXCL)1, CXCL3, CXCL5, CXCL6, and CXCL8 (70). Specifically, IL-17F promotes neutrophil accumulation in the dermis by stimulating the release of IL-8 by keratinocytes (84). T_H17 cytokines (IL-17A, IL-22, and TNF) also cause keratinocytes to produce C-C motif chemokine ligand (CCL) 20 and its receptor, C-C motif chemokine receptor (CCR) 6, thereby facilitating the recruitment and infiltration of T_H17 cells (85). IL-17A-induced keratinocyte production of IL-19 has been shown to upregulate the production of antimicrobial proteins, namely S100 calcium-binding protein (S100)A7, S100A8, and S100A9. IL-19 and IL-17A may also interact synergistically to enhance the keratinocyte response. These observations seem to suggest IL-19 as a potentially novel component of the IL-23/IL-17 axis (86), which plays a crucial role in the development of psoriatic inflammation (87). Apart from its role in T_H17 differentiation and expansion, IL-23 has been demonstrated to induce dermal inflammation and epidermal hyperplasia – mediated through the combined effects of IL-17A (88) and IL-22 (77).

IL-22 is co-expressed with IL-17A and IL-17F by activated T_H17 cells (76, 77) as well as T_H22 cells (89). IL-22 does not affect immune cells (90) and primarily targets epithelial cells, mediating innate immune responses and contributing to wound healing (91). This cytokine has a dual nature, exhibiting both anti- and pro-inflammatory properties (92). IL-22 induces the overexpression of AMPs, such as β-defensin 2, and the S100 protein family (76, 93, 94). In addition, IL-22 has been reported to downregulate the expression of genes involved in the regulation of keratinocyte differentiation, resulting in acanthosis (94–96). As a pro-inflammatory cytokine, IL-22 stimulates keratinocyte production of chemokines (CCL2, CCL20, CXCL5, and CXCL8) (93, 94). These chemotactic agents will promote the infiltration of monocytes/macrophages (97), neutrophils (98), and T cells (99) at sites of cutaneous inflammation. A graphical representation of key cytokine circuits in the immunopathogenesis of PsO is provided in **Figure 1**.

Other cytokines that have been implicated in psoriatic inflammation include IL-9, IL-19, IL-20, IL-24, IL-33, and IL-36.

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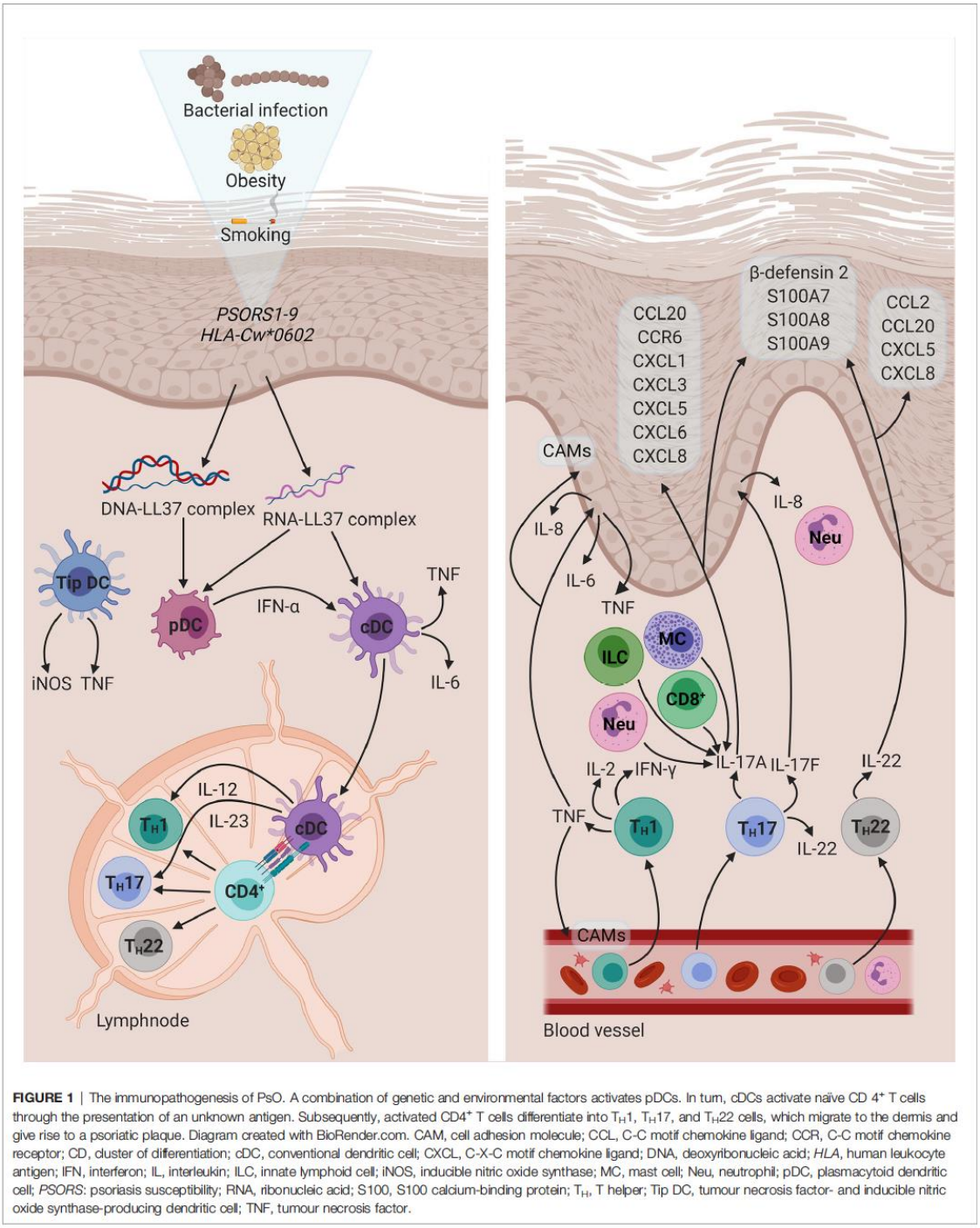

FIGURE 1 | The immunopathogenesis of PsO. A combination of genetic and environmental factors activates pDCs. In turn, cDCs activate naïve CD 4+ T cells through the presentation of an unknown antigen. Subsequently, activated CD4+ T cells differentiate into T_H1, T_H17, and T_H22 cells, which migrate to the dermis and give rise to a psoriatic plaque. Diagram created with BioRender.com. CAM, cell adhesion molecule; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; CD, cluster of differentiation; cDC, conventional dendritic cell; CXCL, C-X-C motif chemokine ligand; DNA, deoxyribonucleic acid; *HLA*, human leukocyte antigen; IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; iNOS, inducible nitric oxide synthase; MC, mast cell; Neu, neutrophil; pDC, plasmacytoid dendritic cell; *PSORS*: psoriasis susceptibility; RNA, ribonucleic acid; S100, S100 calcium-binding protein; T_H, T helper; Tip DC, tumour necrosis factor- and inducible nitric oxide synthase-producing dendritic cell; TNF, tumour necrosis factor.

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IL-9 has been suggested to contribute to PsO pathogenesis *via* its pro-angiogenic activity and induction of IL-17 production (100). In addition, members of the IL-20 subfamily of cytokines (IL-19, IL-20, and IL-24) have been shown to affect keratinocyte proliferation and differentiation and to induce the expression of various PsO-related molecules (101). Indeed, suppression of these cytokines resulted in alleviation of epidermal hyperplasia in psoriatic patients (102). Furthermore, IL-33 was recently demonstrated to act on keratinocytes in an autocrine manner, thereby perpetuating the psoriatic inflammatory response (103). Finally, IL-36 has been implicated in keratinocyte-specific pathways that mediate dermal inflammation in PsO (104, 105).

TREATMENT OF PSORIASIS

When determining the appropriate treatment regimen for a PsO patient, various factors should be taken into consideration, such as disease severity the presence of psoriatic arthritis, comorbidities, and the impact on the patient's quality of life. The severity of PsO may be determined by the percentage of the total body surface area (BSA) that is involved, with <3% BSA considered mild, 3–10% BSA considered moderate, and >10% BSA considered severe. Mild to moderate PsO is treated with topical agents including corticosteroids, calcineurin inhibitors, vitamin D_3 analogues, keratolytic agents, anthralin, retinoids, and coal tar preparations (106). For moderate to severe PsO, phototherapy and systemic treatments are prescribed (107). Oral systemic therapies, such as methotrexate, apremilast, cyclosporine, and acitretin, possess anti-inflammatory and immunomodulatory properties (108). The advent of biologic systemic therapies has drastically changed the treatment of PsO. Currently, there are four classes of biologicals available, namely TNF inhibitors, IL-12/23 inhibitors, IL-17 inhibitors, and IL-23 inhibitors (109). These agents exert their effects by targeting prominent cytokines involved in the pathogenesis of PsO. When compared to traditional systemic agents these therapies have better safety profiles and may also be more efficacious (110).

THE SYSTEMIC INFLAMMATORY NATURE OF PSORIASIS

PsO was initially primarily regarded as a hyperkeratotic disorder solely confined to affected skin areas. However, the systemic inflammatory nature of the condition has become increasingly apparent in recent years (111, 112). Various pro-inflammatory products are overexpressed in psoriatic skin lesions. These mediators also seem to be released into the systemic circulation of psoriatic patients and may reflect disease severity (113). Peripheral inflammation is evident by the abnormal expression of a host of inflammatory molecules in the blood of these individuals (113–116). Moreover, inflammation may also be detected at extracutaneous sites. In a pilot study by Mehta et al. (117), ^{18}F-fluorodeoxyglucose emission tomography-computed tomography was utilised to localise and quantify inflammatory activity in individuals with moderate to severe PsO. The authors detected systemic inflammation in the skin, joints, liver, and vasculature, with significantly greater aortic and hepatic inflammation in psoriatic patients compared to age- and gender-matched controls (117). Furthermore, individuals with PsO have an increased risk of developing multiple comorbid diseases (presented in **Table 1**), which serves as further evidence for the presence of systemic inflammation in the condition. It has been proposed that the chronic course of PsO, as well as common inflammatory molecules and/or pathways, may act as the driving forces of the development and/or worsening of these extracutaneous manifestations (15, 111).

Besides systemic comorbid conditions, individuals with PsO may also develop metabolic abnormalities. An increased prevalence of metabolic syndrome (MetS) – a constellation of dyslipidaemia, hypertension, insulin resistance, and visceral obesity – has been reported in psoriatic patients compared to healthy controls (132, 133, 138–140). However, the directionality of these associations remains unknown. It is possible that PsO may initiate inflammatory pathways that drive the development of metabolic disturbances. Inflamed adipose tissue produces adipocytokines (141) that may worsen existing PsO (142). We note that metabolic dysfunction may also contribute to an increased cardiovascular burden in psoriatic individuals. This is however, beyond the scope of this paper. There are several excellent review articles in which the association between MetS and PsO are discussed (143–145).

THE ASSOCIATION BETWEEN PSORIASIS AND CARDIOVASCULAR DISEASE

In recent years, CVD has emerged as a particularly prominent comorbidity of PsO. Several epidemiological studies have reported an increased risk of CVD in psoriatic individuals, compared to the general population. Furthermore, recent guidelines for the primary prevention of CVD, by the American College of Cardiology and the American Heart Association, indicated PsO as a risk-enhancing factor for the development of atherosclerotic CVD (146).

In a landmark study by Gelfand and colleagues (20), patients with PsO were reported to have a higher incidence of myocardial infarction (MI). Patients with mild and severe PsO had an

TABLE 1 | Comorbidities associated with PsO.

Comorbidity	Selected references
Chronic kidney disease	(118–120)
CVD	(18–20)
Depression	(8, 121, 122)
Diabetes	(123–125)
Inflammatory bowel disease	(126–128)
Malignancy	(129–131)
Metabolic syndrome	(132–134)
Non-alcoholic fatty liver disease	(135–137)

CVD, cardiovascular disease.

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incidence of 4.04 (95% confidence interval [CI]: 3.88–4.21) and 5.13 (95% CI: 4.22–6.17) per 1000 person-years, compared to an incidence of 3.58 in healthy control subjects (95% CI: 3.52–3.65) (20). In addition, traditional CVD risk factors such as hyperlipidaemia, hypertension, and smoking were more prevalent among psoriatic individuals. PsO remained an independent risk factor for CVD, after adjusting for established CVD risk factors. Similar associations were noted in studies assessing the risk of stroke (19) and venous thromboembolism (VTE) (18) in psoriatic individuals. Gelfand et al. (19) reported an increased risk of stroke in mild (hazard ratio [HR] 1.06; 95% CI: 1.0–1.1) and severe (HR 1.43; 95% CI: 1.1–1.9) PsO, after adjusting for major risk factors (19). Furthermore, Ahlehoff and colleagues (18) reported higher incidence rates of VTE in psoriatic individuals (1.92 and 3.20 per 1000 person-years for mild and severe PsO) compared to healthy controls (1.29 per 1000 person-years) (18). In a meta-analysis by Gaeta and colleagues (147), it was shown that PsO confers an excess risk of 24% for the development of CVD (147). It has also been reported that severe disease activity contributes substantially to increased mortality due to CVD (148–150). Mehta et al. (21) identified severe PsO as an independent risk factor for CVD deaths (HR 1.57; 95% CI: 1.26–1.96), after adjusting for traditional CVD risk factors (21). Lastly, in a study by Abuabara and colleagues (149), it was determined that CVD was responsible for the highest absolute (61.9 deaths per 1000 person-years) and excess (3.1 deaths per 1000 person-years) risk in PsO patients (149).

The link between PsO and CVD may potentially be explained by the chronic course of the disease and the associated systemic inflammation. A considerable body of literature supports the notion that chronic low-grade systemic inflammation is a central theme in the pathogenesis and propagation of CVD (151–154). Moreover, elevated levels of C-reactive protein (CRP) – a sensitive marker of systemic inflammation – has been suggested as a predictor of future CVD events (155). Two landmark trials, namely the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study (156), and the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) (157), also provided evidence for a prominent role of systemic inflammation in the etiopathogenesis of CVD. In the following section, we will elaborate on the interplay between inflammation and coagulation as well as how an imbalance in these activities may promote the development of CVD – specifically *via* thrombosis.

PSORIATIC INFLAMMATION AND COAGULOPATHY

Inflammation and coagulation are interdependent processes, demonstrated by the dynamic crosstalk between these systems. Under normal physiological conditions, these systems function as protective mechanisms and are tightly regulated. However, dysregulation may result in chronic, systemic inflammation and/or thrombotic complications. In response to invading pathogens or tissue damage, inflammation ensues to eliminate the original insult and to promote wound healing and tissue repair. However, if the inflammatory process is not duly resolved; acute inflammation may transition to chronic, systemic inflammation. In turn, the sustained activation of the coagulation cascade, driven primarily by pro-inflammatory cytokines, may follow.

A particularly prominent molecule in the context of inflammation-induced coagulation is tissue factor (TF) (25). The tenase complex, comprising TF and factor VIIa, activates the extrinsic coagulation pathway, culminating in the generation of thrombin. In the final steps of coagulation, thrombin catalyses the conversion of soluble fibrinogen to insoluble fibrin. Pro-inflammatory cytokines may upregulate the expression of TF on endothelial cells and monocytes (158). In addition, inflammation favours the suppression of natural anticoagulant mechanisms, namely the antithrombin pathway, the protein C pathway, and tissue factor pathway inhibitor (TFPI) (24, 25). Finally, fibrinolytic activity may also decrease due to a continuous increase in plasminogen activator inhibitor (PAI)-1 levels stimulated by pro-inflammatory molecules (25). Thus, chronic systemic inflammation may alter the haemostatic balance to favour a prothrombotic state.

In turn, coagulation may also modulate and perpetuate the inflammatory response. Coagulation proteases may bind to protease-activated receptors on the activated endothelium, inducing the synthesis and expression of cell adhesion molecules (159, 160). These molecules play a pivotal role in the extravasation of leukocytes to sites of inflammation. Furthermore, activated coagulation factors may also elicit an inflammatory response by interacting with immune cells to induce the production of cytokines (161, 162). Thrombocytes or platelets are also increasingly recognised for their ability to mediate and regulate inflammation. Activated platelets release their granular content, which comprises a plethora of procoagulant and pro-inflammatory molecules. Platelets are also implicated in the recruitment of leukocytes and the regulation of vascular permeability (163).

From these observations, it is evident that inflammation and coagulation should not be viewed as separate entities and that an imbalance in these activities may culminate in the development of prothrombotic conditions. Thrombosis is the most common pathology underlying the three major cardiovascular conditions, namely ischaemic heart disease, ischaemic stroke, and VTE (164–166). Arterial thrombosis, which ensues after the rupture of an atherosclerotic plaque or damage to the vessel wall, may give rise to MI or stroke. Platelets play a central role in the formation of an arterial thrombus (167). In contrast, the pathomechanism of venous thrombosis, associated with deep vein thrombosis (DVT) and pulmonary embolism, is less clear. Virchow's triad describes three factors, namely hypercoagulability, endothelial dysfunction, and altered blood flow, which may predispose an individual to the development of venous thrombosis (168).

As discussed in the previous sections, PsO is characterised by chronic, systemic inflammation and accompanied by an increased risk for CVD. A potential mechanism may be the

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development of a prothrombotic state, *via* the action of multiple pro-inflammatory cytokines, due to psoriatic pathology. The most prominent cytokines that could play a role in the development of a prothrombotic state - IL-6, IL-17, and TNF - will be discussed below. We focus specifically on the effects of these cytokines on platelets and the endothelium. In **Figures 2** and **3** schematic representations are provided of the signalling pathways that could be initiated in platelets and endothelial cells, respectively, upon stimulation with these cytokines.

Interleukin-6

IL-6 is a pleiotropic cytokine involved in several physiological processes including the acute-phase response and antibody production (169). The effects of IL-6 are mediated *via* two receptors, namely the IL-6 receptor (IL-6R) and the signal-transducing subunit, glycoprotein (gp) 130 (169). In PsO, IL-6, combined with IL-23, contributes to the differentiation of T_H17 cells (12). The overexpression of this cytokine has been detected in psoriatic skin lesions (170) as well as in the circulation of these individuals (113, 171, 172). In addition, IL-6 signalling has been suggested to diminish regulatory T cell activity in PsO, thereby allowing the expansion of effector T cells (170). In the context of CVD, IL-6 has emerged as a pivotal mediator of thrombotic disease. Raised IL-6 levels have been found to be related to recurrent venous thrombosis (173), with detectable levels of IL-6 associated with a two-fold increase in the risk of venous thrombosis (174). The potential involvement of IL-6 in the development of adverse cardiovascular events is further supported by findings of a sub-study of the CANTOS trial. In this study, baseline IL-6 levels were associated with an increased risk of cardiovascular events. More importantly, it was demonstrated for the first time that lowering IL-6 levels – *via* the inhibition of IL-1β by canakinumab – resulted in a 15% reduction of atherothrombotic events (MI and stroke) (175).

During inflammation, IL-6 acts on hepatocytes to induce the synthesis of acute-phase proteins such as CRP, fibrinogen, and serum amyloid A (SAA) (176). Both CRP and SAA have demonstrated the ability to promote coagulation *via* the induction of TF synthesis in endothelial cells and suppression of the TFPI pathway (177, 178). With regard to fibrinogen, it has been suggested that a causal relationship exists between hyperfibrinogenaemia and thrombosis (179).

Although platelets do not express IL-6R, they have been shown to express gp130 (180, 181). Upon thrombin-induced platelet activation, the soluble form of IL-6R is secreted which can form a complex with IL-6 (180, 181). This complex may activate gp130, in a process termed trans-signalling, resulting in the activation of signal transducer and activator of transcription (STAT) 3 (181). However, IL-6 trans-signalling does not seem to affect platelet activation and/or aggregation. Nevertheless, IL-6 may promote platelet adhesion and aggregation indirectly. IL-6 inhibits the activity of a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13 (ADAMTS13) (182). ADAMTS13 is responsible for the cleavage of ultra-large von Willebrand factor multimers released by the endothelium. In the ultra-large form, these multimers are hyperreactive and may interact with platelets to induce adhesion and aggregation.

Interleukin-17

IL-17 plays a pivotal role in the innate immune response, particularly in host defence against microbial invasion (183). The IL-17 family consists of six members, namely IL-17A to IL-17F, and signalling is mediated through the IL-17 receptor (IL-17R) family that comprises five members, IL-17RA to IL-17RE (184). This pro-inflammatory cytokine has been implicated in the pathogenesis of diverse autoimmune and inflammatory diseases, including PsO (185). Indeed, IL-17 is regarded as the principal driver of psoriatic inflammation. The overexpression of this cytokine has been detected in lesional psoriatic skin, when compared to nonlesional skin (186, 187). In addition, serum levels of IL-17A are elevated in psoriatic patients (188) and may also reflect disease severity (186). With respect to cardiovascular pathology, conflicting results have been reported on the role of IL-17. Some studies have found a protective effect of IL-17 (189), while others have reported a pro-atherogenic role for IL-17 (190). Nevertheless, prothrombotic effects of IL-17 have been demonstrated. In a murine model of DVT, IL-17A was found to promote thrombus formation by enhancing platelet aggregation and neutrophil infiltration of thrombi (191). Moreover, it has been shown that targeted blocking of IL-12/23 and IL-17 resulted in improved skin phenotype and lengthened clotting times to occlusive thrombus formation in a murine model of psoriatic disease (192).

Platelets have been demonstrated to express a functional IL-17 receptor, namely IL-17RA (193). Exposing activated platelets (induced by adenosine diphosphate) to IL-17 augments platelet aggregation (193–195). This action is suggested to be mediated by the opening of the mitochondrial permeability transition pore (196) and the phosphorylation of extracellular signal-regulated kinase-2 (194, 196). Furthermore, stimulation with IL-17 causes an increased expression and accelerated externalisation of P-selectin and exposure of the $\alpha_{IIb}\beta_3$ integrin (193, 194, 196). Both of the aforementioned molecules facilitate platelet aggregation and are used as markers of platelet activation.

IL-17 may also alter the endothelium towards an activated state. IL-17RA is constitutively expressed in endothelial cells (197). It has been reported that IL-17 and TNF act synergistically to activate the endothelium, resulting in the synthesis of inflammatory mediators, and the expression of cell adhesion molecules (E-selectin and intercellular adhesion molecule-1) and TF by these cells (195). Anticoagulant activity by the endothelium also becomes diminished, as thrombomodulin expression is downregulated (195). It should be noted that IL-17 alone is not a strong inducer of inflammatory activity, however, IL-17 in concert with TNF potently induce pro-inflammatory gene expression. This effect is thought to be mediated by the ability of IL-17 to stabilise messenger RNA (198, 199). Finally, the vascular expression of CD39, an inhibitor of platelet aggregation, is also downregulated by IL-17A (195, 200).

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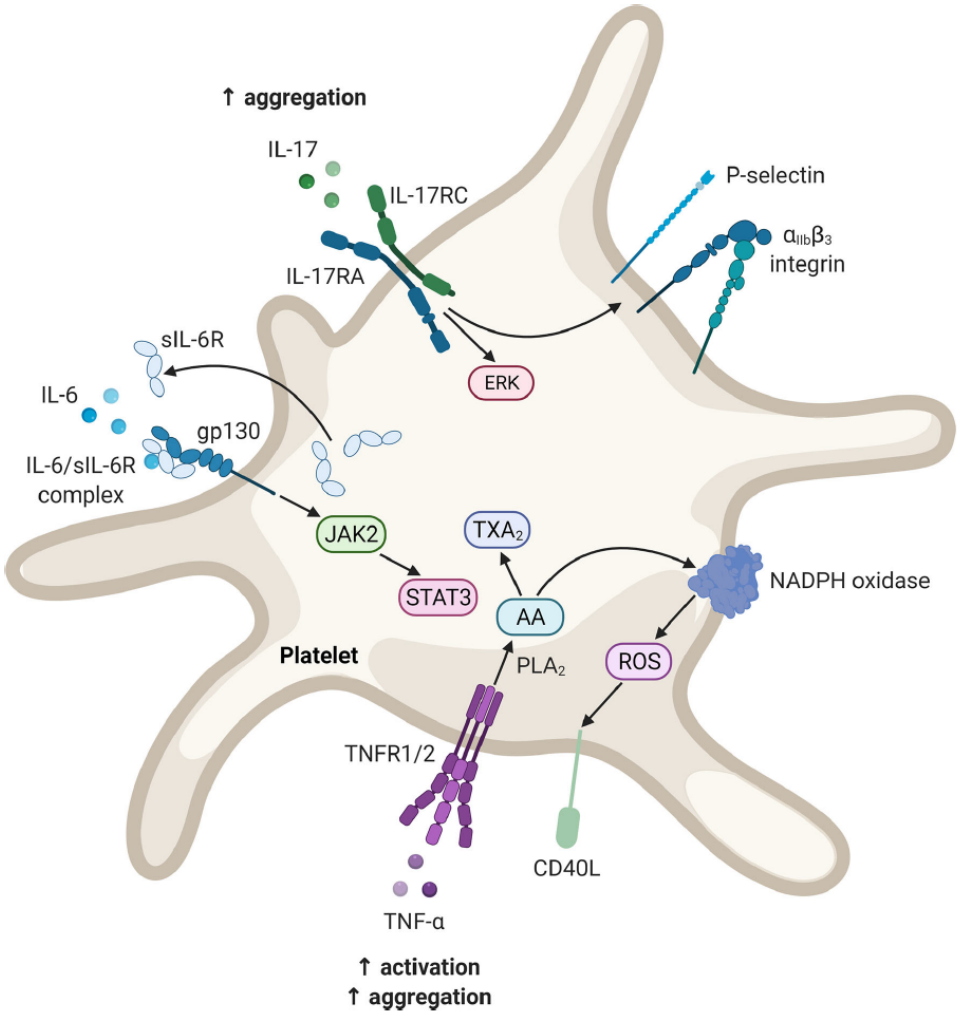

FIGURE 2 | Potential signalling pathways in platelets upon stimulation with IL-6, IL-17, and TNF. These mediators may contribute to platelet activation as well as enhancing the platelet response to agonist-induced platelet activation. Diagram created with BioRender.com. AA, arachidonic acid; CD40L, CD40 ligand; ERK, extracellular signal-regulated kinase; gp, glycoprotein; IL, interleukin; IL-17RA, interleukin-17 receptor A; IL-17RC, interleukin-17 receptor C; JAK, Janus kinase; NADPH, nicotinamide adenine dinucleotide phosphate; PLA_2, phospholipase A_2; ROS, reactive oxygen species; sIL-6R, soluble interleukin-6 receptor; STAT, signal transducer and activator of transcription; TXA_2, thromboxane A_2; TNF, tumour necrosis factor; TNFR, tumour necrosis factor receptor.

Tumour Necrosis Factor

TNF is a potent inflammatory cytokine, orchestrating various processes such as inflammation, cell differentiation, and apoptosis (201). The effects of TNF are mediated by two receptors, namely TNF receptor (TNFR) 1 and TNFR2 (202). In PsO, this cytokine amplifies the inflammatory response *via* the generation of reactive oxygen species and inducing the expression of cytokines and cell adhesion molecules (73, 203). Increased TNF activity has been detected in involved skin from psoriatic individuals (204) and TNF levels were also elevated in the serum of these individuals (113, 205). In relation to cardiovascular diseases, increased concentrations of TNF have been associated with recurrent coronary events (206), and venous thrombosis (174). In addition, TNF levels have been

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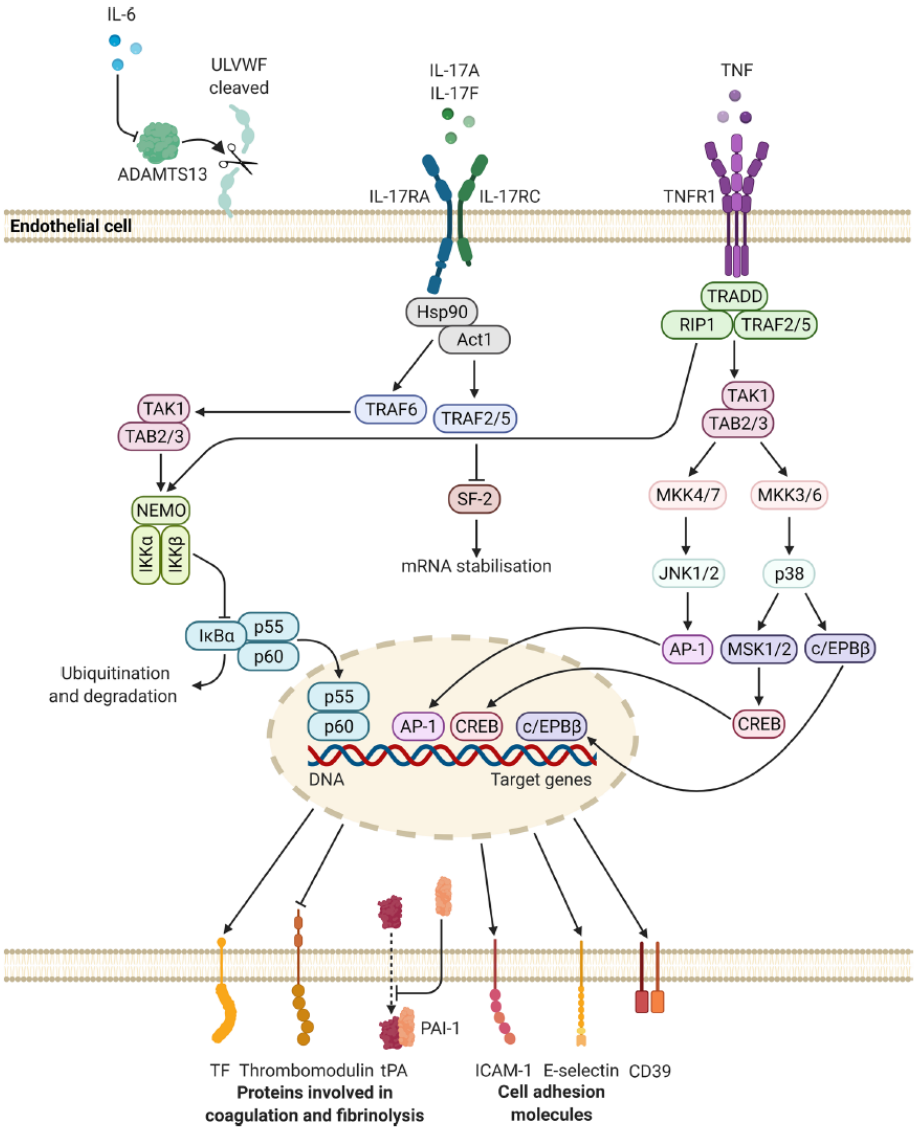

FIGURE 3 | Potential intracellular signalling pathways initiated in endothelial cells, upon stimulation with IL-6, IL-17, and TNF. These mediators may upregulate the synthesis and release of molecules that promote platelet adhesion and coagulation, while suppressing the expression of proteins involved in fibrinolysis. Altogether these changes may lead to a prothrombotic state. Diagram created with BioRender.com. ADAMTS13, a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13; AP, activator protein; c/EPB, CCAAT/enhancer-binding protein; CD, cluster of differentiation; CREB, cAMP response element binding protein; DNA, deoxyribonucleic acid; Hsp, heat shock protein; ICAM-1, intercellular adhesion molecule-1; IKBα, NF-kappa-B inhibitor alpha; IKK, inhibitor of nuclear factor kappa-B kinase; IL, interleukin; IL-17RA, interleukin-17 receptor A; IL-17RC, interleukin-17 receptor C; JNK, c-Jun N-terminal kinase; MKK, mitogen-activated protein kinase kinase; MSK, mitogen- and stress-activated protein kinase; NEMO, NF-kappa-B essential modulator; PAI-1, plasminogen activator inhibitor-1; SF, splicing factor; TF, tissue factor; TNF, tumour necrosis factor; TNFR, tumour necrosis factor receptor; tPA, tissue-type plasminogen activator; TRADD, tumour necrosis factor receptor 1-associated death domain protein; TRAF, tumour necrosis factor receptor-associated factor; RIP, receptor-interacting protein kinase; ULVWF, ultra-large von Willebrand factor.

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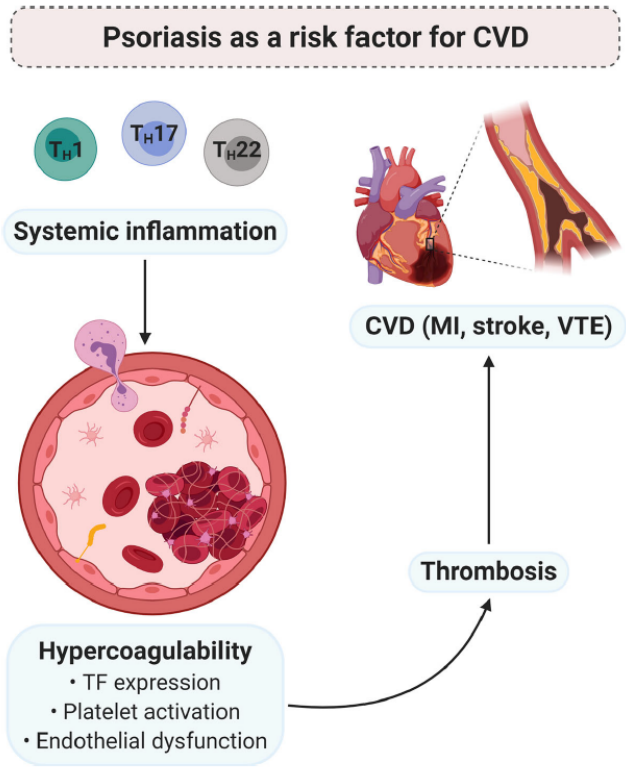

FIGURE 4 | PsO as a risk factor for CVD. Chronic low-grade systemic inflammation, due to psoriatic pathology, may contribute to the development of a hypercoagulable state. Pro-inflammatory mediators induce the expression of TF, enhance platelet activation and/or the platelet response, and endothelial dysfunction. In turn, hypercoagulability predisposes thrombosis, which is the major underlying pathology of CVD. Diagram created with BioRender.com. CVD, cardiovascular disease; MI, myocardial infarction; TF, tissue factor; T_H, T helper; VTE, venous thromboembolism.

suggested to be an independent predictor of cardiovascular events such as MI, stroke, and CVD mortality (207). Regarding the prothrombotic properties of TNF, discrepant results have been reported. In a murine model of atherothrombosis, potent antithrombotic effects of TNF were reported (208). In contrast, another study found that treatment with this cytokine resulted in accelerated thrombus formation (209).

It has been suggested that TNF may promote the (hyper) activation of platelets by interacting with TNFR1 and TNFR2, which is expressed on the platelet membrane (210). TNF may also facilitate platelet activation by stimulating the arachidonic acid pathway (210). Furthermore, CD40 ligand (CD40L) expression by activated platelets may be induced by TNF *via* an arachidonic acid-dependent oxidative stress mechanism (211, 212). In turn, CD40L enhances platelet activation and aggregation, as well as thrombus formation (213). TNF also induces the release of large von Willebrand factor multimers from endothelial cells, which propagates platelet thrombus formation (182). Nevertheless, the ability of TNF to stimulate platelet activation directly remains contested, with some studies reporting no effect (209).

TNF may alter the properties of the endothelium by inducing the synthesis of procoagulant molecules and suppressing natural anticoagulant mechanisms. Engagement of TNF with TNFR1 results in the expression of TF on the surface of endothelial cells and the subsequent deposition of fibrin (209, 214–216). In addition, the production of platelet-activating factor (PAF), by the endothelium, is also induced by TNF (217, 218). PAF induces platelet aggregation (219) and functions as a potent inflammatory mediator (220). The activity of the anticoagulant molecule, activated protein C, is dependent on the presence of functional endothelial thrombomodulin, which has been shown to be downregulated by TNF (216). Mechanistically, this inhibitory effect is mediated by downregulating the transcription of the

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thrombomodulin gene (209, 221). Finally, TNF inhibits fibrinolysis by induction of the PAI-1 gene (209) through the activity of NF-κB (222) and by decreasing the release of tPA (223).

CONCLUDING REMARKS

PsO is an immune-mediated inflammatory disorder of the skin, characterised by the overexpression of T_H1-, T_H17-, and T_H22-derived inflammatory cytokines. These mediators are upregulated in the lesional skin of psoriatic individuals and may also be released into the circulatory system of these patients. The systemic inflammatory nature of the condition is reflected by a multitude of dysregulated inflammatory molecules, which may give rise to various comorbidities. PsO has been identified as an independent risk factor for the development of CVD (**Figure 4**). The chronic subclinical systemic inflammation associated with the condition may predispose psoriatic individuals to thrombosis – a major determinant of CVD. Inflammatory cytokines may contribute to the development of a prothrombotic state, *via* the induction of TF, platelet activation and/or enhancing the platelet response, and endothelial dysfunction.

The exact mechanisms underlying the association between PsO and CVD remains elusive. However, it is imperative that both physicians and patients must be aware of the potential cardiovascular risk that PsO may pose. Therefore, effective management of the condition should not only aim to ameliorate cutaneous inflammation but also systemic inflammation, in order to prevent secondary comorbidities such as CVD. Finally, as pointed out in a recent review by Aksentijevich and colleagues (224), PsO presents a unique opportunity as a human model for the study of chronic systemic inflammation in the development of CVD (224). Treatment of psoriatic patients with targeted biological therapies may shed light on the contribution of specific cytokines to cardiovascular morbidity, and provide novel treatment targets.

AUTHOR CONTRIBUTIONS

MV: writing of paper, and preparation of figures. GT: editing of paper. EP: study leader, corresponding author, editing of paper, funding. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix D: Publication

scientific reports

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Psoriatic disease is associated with systemic inflammation, endothelial activation, and altered haemostatic function

Maria J. E. Visser[1], Chantelle Venter[1], Timothy J. Roberts[1,2,3], Gareth Tarr[1,4] & Etheresia Pretorius[1]✉

Psoriasis is a chronic, immune-mediated inflammatory skin disease, affecting approximately 2% of the general population, which can be accompanied by psoriatic arthritis (PsA). The condition has been associated with an increased cardiovascular burden. Hypercoagulability is a potential underlying mechanism that may contribute to the increased risk of major cardiovascular events in psoriatic individuals. Whole blood samples were collected from 20 PsA patients and 20 healthy individuals. The concentrations of inflammatory molecules (C-reactive protein, serum amyloid A, soluble intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1, and soluble P-selectin) were determined by enzyme-linked immunosorbent assays. In addition, clotting efficiency was evaluated by thromboelastography. The fibrin network architecture was also assessed by scanning electron microscopy. Elevated levels of circulating inflammatory molecules were significantly associated with the presence of psoriatic disease. Furthermore, an increased tendency towards thrombus formation was significantly predictive of disease presence. Scanning electron microscopy revealed that fibrin clots were denser in psoriatic individuals, compared to healthy controls, with an increased fibrin fibre diameter associated with psoriatic disease. Our results add to the accumulating evidence of the systemic nature of psoriasis and the subsequent risk of cardiovascular comorbidities, potentially due to an acquired hypercoagulability. We suggest that haemostatic function should be monitored carefully in psoriatic patients that present with severe disease, due to the pre-eminent risk of developing thrombotic complications.

Psoriasis is a chronic, inflammatory skin disease, affecting approximately 2% of the global population[1]. Disease development is a complex interplay, involving genetic predisposition, environmental exposures, and disordered innate and adaptive immune responses. The condition commonly manifests as erythematous, well-demarcated plaques covered by slivery-white scales, and up to 30% of affected individuals could develop an inflammatory arthritis [psoriatic arthritis (PsA)][2]. The cytokine network in psoriasis is primarily polarised towards the over-expression of T helper (T_H) 1 and T_H17 cytokines[3]. Prominent inflammatory mediators implicated in the initiation and maintenance of the disease, include interferon (IFN)-α, interleukin (IL)-22, the IL-23/IL-17 axis, and tumour necrosis factor (TNF)-α[4–7]. Chronic, subclinical systemic inflammation is evidenced by elevated levels of these and other inflammatory molecules in the blood of psoriatic patients. Individuals with psoriasis are also at an increased risk, when compared to the general population, of developing comorbidities such as depression, diabetes, inflammatory bowel disease, malignancy, metabolic syndrome, and cardiovascular disease (CVD)[8–13]. It has been proposed that shared inflammatory pathways may act as a driving force for both psoriasis and its extracutaneous manifestations[14]. Considering these observations, a paradigm shift has occurred from viewing psoriasis as merely 'skin-deep' to a systemic inflammatory condition[15]. A new concept, namely 'psoriatic disease', has also been introduced to describe skin and joint manifestations as well as the involvement of various other

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organ systems in the same individual[16]. Recently, the term 'psoriatic syndrome' has been proposed to rather define the condition as a syndrome that comprises diverse clinical features that may or may not occur at different stages of the disease[17].

In recent years, CVD has been recognised as a prominent comorbidity of the condition. Various epidemiological studies have reported a significantly increased risk for major cardiovascular events, such as myocardial infarction (MI)[18], stroke[19], and venous thromboembolism (VTE)[8], in individuals with psoriasis. Moreover, the presence of psoriasis has been identified as an independent risk factor for the development of CVD after adjusting for traditional risk factors[18–20]. Similarly, PsA also confers an increased risk for the development of cardiovascular events, such as MI and stroke[21]. It has been shown that the 10-year risk of adverse cardiac events, as determined by the Framingham Risk Score, is underestimated in these individuals[22]. Moreover, it has been recommended by the European League Against Rheumatism (EULAR) task force that CVD risk should be evaluated regularly in PsA patients, while also aiming to manage disease activity to lower the associated risk[23].

Thrombophilia or hypercoagulability might be a potential mechanism underlying the relationship between psoriasis and CVD. The processes of inflammation and coagulation are interconnected, with these systems interacting in a bidirectional manner. A hallmark of sustained, low-grade systemic inflammation is a shift in the haemostatic balance towards a prothrombotic state[24]. Elevated levels of C-reactive protein (CRP), an acute-phase reactant and marker of inflammation, have been shown to be predictive of an increased risk of developing thrombotic disease[25–27]. Pro-inflammatory cytokines are the major mediators of inflammation-induced coagulation activation. These molecules disturb the haemostatic balance by inducing the expression of tissue factor (TF) on endothelial cells and monocytes, downregulating endogenous anticoagulant mechanisms, and impairing fibrinolytic activity[28,29]. TF plays a central role in coagulation, as it functions as a cofactor for factor VIIa in the extrinsic tenase complex. This complex initiates the extrinsic pathway of coagulation, resulting in the generation of thrombin. In the terminal stages of coagulation, soluble fibrinogen is converted into insoluble fibrin through the enzymatic action of thrombin. Fibrin plays a fundamental role in haemostasis, providing the structural scaffolding for blood clots. The fibrin network architecture is an important determinant of clot stability and fibrinolytic susceptibility[30]. Compact, less permeable clots, consisting of thin fibrin fibres, are associated with an increased risk of thrombotic events. This prothrombotic fibrin clot phenotype has been associated with various thromboembolic diseases, such as coronary artery disease[31], stroke[32], and VTE[33]. Therefore, characterisation of the fibrin network architecture might be a useful biomarker for thrombosis.

The vascular endothelium serves as an important interface between inflammation and coagulation, playing an essential role in the regulation of these entities. Under physiological conditions, the intact endothelium exhibits anti-inflammatory properties and expresses anticoagulant proteins[34,35]. However, upon stimulation with pro-inflammatory molecules, endothelial cells become activated[36]. Consequently, the endothelium upregulates the expression of cell adhesion molecules (CAMs) [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)], selectins (E-selectin and P-selectin), inflammatory mediators, and procoagulant factors, while attenuating the expression of anticoagulants. This functional deterioration of the endothelial barrier could exert a net prothrombotic effect. Additionally, the inflamed endothelium could promote the firm adhesion and full activation of platelets via the interactions between CAMs and/or selectins and platelet surface receptors[37,38]. The interaction of P-selectin with its receptor, P-selectin glycoprotein ligand-1, is a key mediator of endothelial-platelet interactions[39] and the formation of platelet-leukocyte aggregates[37]. Impaired endothelial function and platelet hyperactivity have been implicated in the pathogenesis of arterial and venous thrombosis[40,41].

In this paper, we investigated whether a hypercoagulable state is present in psoriatic patients, compared to healthy individuals. To this end, we evaluated whole blood (WB) coagulation efficiency and characterised the fibrin network architecture. In addition, the levels of biomarkers indicative of inflammation, endothelial dysfunction, and platelet activation were measured.

Methods

Ethical clearance and informed consent. This study received ethical approval from the Health Research Ethics Committee (HREC) of Stellenbosch University, Stellenbosch, South Africa (HREC reference N19/03/043). Prior to WB collection, a written form of informed consent was obtained from all study participants. All methods were carried out in accordance with the guidelines of the relevant ethics committees. We strictly adhered to the Declaration of Helsinki.

Study design and study population. A cross-sectional study design was followed. The study population consisted of n = 40 volunteers, which included n = 20 PsA patients and n = 20 healthy individuals. Psoriatic patients were recruited during their visits to the Winelands Rheumatology Centre, Stellenbosch, for routine consultations. All psoriatic patients were assessed by the same rheumatologist. Psoriasis skin severity was assessed using the Psoriasis Area and Severity Index (PASI) score. For PsA, disease severity was assessed using the Disease Activity in PSoriatic Arthritis (DAPSA) score. According to DAPSA scores, PsA disease activity was classified as low, moderate, or high. Enthesitis was graded using the Leeds Enthesitis Index, while joint involvement was assessed by the 66/68 joint count for swollen and tender joints. Inclusion criteria for psoriatic patients were as follows: (1) fulfilled ClASsification of criteria for Psoriatic ARthritis (CASPAR) (see Table 1); (2) presence of any clinical variant of psoriasis; and (3) psoriatic patients were allowed to be on some form of systemic treatment. Corticosteroid usage included cortisone, methylprednisolone, and prednisone. Treatment with disease-modifying anti-rheumatic drugs (DMARDs) included leflunomide, methotrexate, and sulfasalazine. Biological agents included adalimumab (Humira), etanercept (Enbrel), infliximab (Revellex), and upadacitinib. Age-matched healthy individuals with no history of psoriasis or inflammatory disease were recruited. WB were collected at

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Criterion	Description
1. **Evidence of psoriasis**	
Current psoriasis	Psoriatic skin or scalp disease as judged by rheumatologist or dermatologist
Personal history of psoriasis	History of psoriasis that may be obtained from patient, family physician, dermatologist, rheumatologist
Family history of psoriasis	History of psoriasis in first- or second-degree relative according to patient
2. **Psoriatic nail dystrophy**	Onycholysis, pitting, and hyperkeratosis
3. **Negative test for rheumatoid factor**	Determined by any method, except latex, but preferably by enzyme-linked immunosorbent assay or nephelometry; based on reference range of local laboratory
4. **Dactylitis**	
Current dactylitis	Swelling of entire digit
History of dactylitis	History as recorded by rheumatologist
5. **Radiographic evidence of juxtaarticular new bone formation**	Ill-defined ossification near joint margins (excluding osteophyte formation) on radiographs of hands or feet

Table 1. CASPAR criteria[75] for the diagnosis of PsA. A patient must have inflammatory articular disease (joint, spine, or entheseal) with a score of ≥ 3 points of the following 5 criteria. Current psoriasis is assigned a score of 2, while all other features are assigned a score of 1.

the Department of Physiological Sciences, Stellenbosch University, by a Medical Biological Scientist and phlebotomist registered with the Health Professionals Council of South Africa (MW: 0,010,782). Exclusion criteria for all study participants were as follows: (1) suffering from a known chronic inflammatory condition(s), namely human immunodeficiency virus, malignancies, and/or tuberculosis; (2) smoking; (3) using anticoagulant and/or antiplatelet medication; and (4) females using contraceptive medication or hormone replacement therapy.

Collection of whole blood and preparation of platelet-poor plasma. A qualified nurse or phlebotomist collected WB from an antecubital vein via venipuncture, using standard sterile techniques. WB was collected in three 4.5 mL BD Vacutainer Citrate Tubes with 3.2% buffered sodium citrate solution (369714, Becton, Dickinson and Company, Franklin Lakes, NJ, USA,). Tubes were left at room temperature for at least 30 min before experiments were performed. Sample processing was completed within 24 h of blood collection. Platelet-poor plasma (PPP) was prepared by centrifuging WB at 3000 × *g* for 15 min at room temperature. Afterwards, PPP was aliquoted and stored at − 80 °C until further laboratory analysis.

Thromboelastography. Thromboelastography (TEG) is a non-invasive viscoelastometric method which measures the ability of a blood sample to form a clot in a quantitative manner. TEG was performed, using the TEG 5000 Hemostasis Analyzer System (07-033, Haemonetics, Boston, MA, USA), to assess the clot kinetics and viscoelastic properties of naive (untreated) WB samples from psoriatic patients and healthy controls. Samples were prepared as follows: 20 µL of 0.2 M calcium chloride ($CaCl_2$) (7003, Haemonetics) was added to a disposable TEG cup (6211, Haemonetics), followed by the addition of 340 µL of WB. $CaCl_2$ was added to reverse the anticoagulant action of sodium citrate and consequently, activate the coagulation cascade. Samples were loaded in the measuring channels of the TEG and analyses were performed at 37 °C. Refer to Table 2 for a brief explanation of the seven TEG parameters that were assessed in this study.

Scanning electron microscopy. Fibrin clots were prepared from PPP for ultrastructural analysis of fibrin fibres by scanning electron microscopy (SEM). To create a fibrin fibre network, 10 µL of PPP was deposited on a 10 mm round glass coverslip and 5 µL of human thrombin (provided by the South African National Blood Service, final concentration 7 IU/mL) was added. Subsequently, samples were covered with 1X Gibco phosphate-buffered saline (PBS), pH 7.4 (10010015, Thermo Fisher Scientific, Waltham, MA, USA) for at least 15 min. Afterwards, primary fixation was performed by covering samples with 4% formaldehyde (158127, Sigma-Aldrich, St. Louis, MO, USA), which cross-links proteins, for a minimum of 30 min. Thereafter, samples were washed three times for 3 min with PBS. Secondary fixation was performed with 1% osmium tetra-oxide (OsO_4) (75632, Sigma-Aldrich), which cross-links lipids, for 15 min. Afterwards, samples were washed three times for 3 min with PBS. Samples were serially dehydrated with increasing concentrations of ethanol, 30%, 50%, 70%, 90% and three times with 100% ethanol, for 3 min each. A dehydration step was performed by covering samples with 99.9% hexamethyldisilazane (HMDS) (379212, Sigma-Aldrich) for 30 min. HMDS was removed and a final drop of HMDS was added directly to the sample, whereafter samples were left to air-dry overnight in a flow-hood. Coverslips were mounted with double-sided carbon tape on glass microscope slides and sputter coated with carbon, using a Quorum Q150T E Plus carbon coater (Quorum Technologies, Laughton, East Sussex, UK). Samples were examined using the Zeiss MERLIN Field Emission Scanning Electron Microscope (Carl Zeiss, Oberkochen, Germany), housed at the Central Analytical Facilities Electron Microscopy Unit, Stellenbosch University. Electron micrographs were captured with the high resolution InLens detector at 1 kV.

Micrographs of a subset of psoriatic patients (n = 9) and healthy controls (n = 9) were identified for further analysis of fibrin fibre diameter, using ImageJ (version 1.52a). A grid was overlaid on these micrographs,

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Parameter	Unit of measurement	Interpretation
Reaction time (R)	min	**Activation phase:** Time from start of test to first detectable fibrin formation (amplitude of 2 mm). Influenced by concentration of coagulation factors.
Kinetics (K)	min	**Amplification phase:** Time taken to form a clot with a certain level of strength (amplitude of 20 mm). Influenced by fibrinogen concentration and to a lesser extent, platelet function.
Alpha angle (A)	deg	**Propagation phase:** The angle measures the maximal speed of thrombin generation and fibrin formation and cross-linking. Influenced by fibrinogen concentration and to a lesser extent, platelet function.
Maximum amplitude (MA)	mm	**Termination phase:** Maximum mechanical strength/stiffness of clot. Influenced by fibrin cross-linking, platelet count and platelet glycoprotein IIb/IIIa interactions.
Maximum rate of thrombus generation (MRTG)	dynes/cm²/s	First derivative of the velocity of the increase in clot strength based on the change in the elastic modulus, G, where G = (5000MA)/(100-MA).
Time to maximum rate of thrombus generation (TMRTG)	min	Time interval observed before maximum speed of clot growth.
Total thrombus generation (TTG)	dynes/cm²	Total area under the velocity curve, representing the clot strength generated during clot growth.

Table 2. TEG clot parameters for WB[57,76,77].

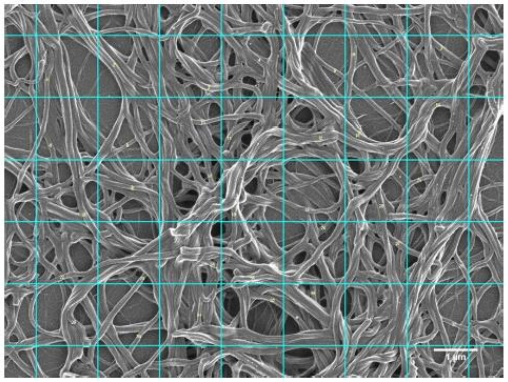

Figure 1. Representative scanning electron micrograph with grid overlay. Numbered fibres indicate fibres that were measured. Individual grid blocks represent an area of 2 μm². Total grid area is 97 μm² (scale bar = 1 μm).

consisting of 5 vertical blocks and 7 horizontal blocks. Each 1.4 μm × 1.4 μm grid block measured an area of 2 μm². In each block of the grid, a fibrin fibre was selected randomly, and the fibre diameter measured (Fig. 1). For each of the individuals included in the subset, three micrographs were analysed, with 35 fibres measured per micrograph. Therefore, 105 measurements were taken per individual. In total, 945 measurements were taken in both the patient and control groups. In order to graphically illustrate differences in the distribution of fibrin fibre diameter, in healthy controls and psoriatic patients, frequency bar graphs were constructed. Additionally, differences in the minimum, maximum, and mean fibre diameter were evaluated between groups.

Soluble P-selectin. The concentration of soluble (s)P-selectin/CD62P was determined using the Human sSELP (Soluble P-Selectin) ELISA kit (EH3818, Fine Biotech Co., Ltd., Wuhan, Hubei, China). Prior to analysis, PPP from psoriatic patients and healthy controls were thawed from −80 °C to room temperature. PPP was diluted 250X with the supplied Sample Dilution Buffer. Before samples were added, the supplied ELISA microplate (pre-coated with capture antibodies) was washed two times with Wash Buffer. Subsequently, 100 μL of sample, standard or control was added per well and incubated for 1.5 h at 37 °C. After incubation, the plate was washed two times with Wash Buffer. Thereafter, 100 μL of biotin-labelled antibody was added per well and incubated at 37 °C for 1 h. The plate was washed again three times with Wash Buffer. Subsequently, 100 μL of the horseradish peroxidase (HRP)-streptavidin conjugate was added per well and incubated for 30 min at 37 °C. Streptavidin binds to biotin with high affinity. After washing the plate five times with Wash Buffer, 90 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was added per well and incubated in the dark for 10 min at 37 °C. TMB is a colorimetric substrate which reacts with HRP. Finally, 50 μL of Stop Solution was added per well, resulting in the formation of a yellow reaction product. The absorbance was read at a wavelength of 450 nm. Samples were analysed in duplicate.

Vascular injury panel. Biomarker analysis was performed using the V-PLEX Vascular Injury Panel 2 (human) Kit (K15198D, Meso Scale Diagnostics, Rockville, MD, USA). This kit measures four biomarkers associated with acute inflammation and tissue damage, namely CRP, serum amyloid A (SAA), soluble (s)ICAM-1/

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Demographics	Healthy individuals (n = 20)	Psoriatic patients (n = 20)
Age, years	58.15 ± 2.785	54.25 ± 2.148
Gender		
Female, n (%)	6 (30)	8 (40)
Male, n (%)	14 (70)	12 (60)
Comorbidities		
Anaemia, n (%)		1 (5)
Diabetes, n (%)		5 (25)
Hypercholesterolaemia, n (%)		4 (20)
Hypertension, n (%)		9 (45)
Hypothyroidism, n (%)		2 (10)
Ischaemic heart disease, n (%)		3 (15)
Menopause, n (%)		1 (5)
Disease activity		
DAPSA		
Low, n (%)		4 (20)
Moderate, n (%)		7 (35)
High, n (%)		9 (45)
Dactylitis, n (%)		7 (35)
PASI		5.300 (1.075–14.85)
Treatment		
Topical corticosteroids, n (%)		3 (15)
DMARDs, n (%)		12 (60)
Biologic agent, n (%)		4 (20)

Table 3. Demographic features and clinical characteristics of healthy individuals and psoriatic patients. Normally distributed data is expressed as mean ± standard error of the mean and non-normally distributed data is expressed as median and (25–75% quartile range).

CD54, and soluble (s)VCAM-1/CD106. Prior to analysis, PPP from psoriatic patients and healthy control subjects were thawed from − 80 °C to room temperature. PPP was diluted 1000X with the supplied MSD Diluent, as recommended by the manufacturer. Before samples were added, the supplied MSD MULTI-SPOT 96-Well Spot plate was washed three times with MSD Wash Buffer. Subsequently, 25 µL of sample, calibrator or control was added per well (precoated with capture antibodies) and incubated for 2 h at room temperature. After incubation, the plate was washed again three times with MSD Wash Buffer. Thereafter, 25 µL of detection antibody was added per well and incubated for 1 h. Detection antibodies (MSD SULFO-TAG) are conjugated with electrochemiluminescent labels. The plate was washed one final time with MSD Wash Buffer and 150 µL of MSD Read Buffer was added to each well. The plate was read on the MSD Discovery Workbench 4 instrument by applying a voltage, which causes the emittance of light. The intensity of emitted light is proportional to the amount of analyte present in the sample. The MSD DISCOVERY WORKBENCH software was used to acquire and analyse data. Samples were analysed in duplicate.

Statistical analysis. R version 4.0.3 was used to perform logistic regression to determine the strength of associations between study variables and disease status (presence or absence of psoriatic disease). More specifically, logistic regression was performed on covariates directly (Model 1) and with adjustment for age and gender (Model 2). Odds ratios (ORs) are reported with 95% confidence intervals (CIs). GraphPad Prism version 8.4.3 was used to produce summaries and plots of the data. In order to summarise differences between groups, an unpaired t-test was performed for normally distributed data (as determined by the Shapiro Wilk normality test), while the Mann–Whitney test was performed for non-normally distributed data. Normally distributed data is expressed as mean ± standard error of the mean and non-normally distributed data is expressed as median and (25–75% quartile range). A p-value of less than 0.05 was considered to be statistically significant. A single missing TEG K value was imputed, using the mean of the K values of the control population.

Results

Demographic features and clinical characteristics of healthy individuals and psoriatic patients are presented in Table 3. There were no significant differences in the age and gender between the study groups. According to DAPSA scores, 4 patients presented with low disease activity, 7 with moderate disease activity, and 9 with high disease activity. Dactylitis was present in 7 patients. The median PASI score was 5.300, representing moderate disease severity.

Biomarker analysis was performed to determine the levels of specific inflammatory molecules in the PPP of healthy individuals and psoriatic patients. Box-and-whisker plots, showing the distribution of these parameters in healthy individuals and psoriatic individuals, are shown in Fig. 2. CRP, SAA, and sICAM-1 levels were

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significantly elevated in the psoriatic group when compared to controls (Fig. 2). No significant differences were noted in the sVCAM-1 levels when psoriatic individuals were compared to control subjects. Logistic regression models indicated that elevated levels of acute-phase reactants, namely CRP (OR 1.402 CI 1.146–1.838) and SAA (OR 1.144 CI 1.038–1.315), were significantly associated with the presence of psoriatic disease (Table 4). Elevated concentrations of these biomarkers indicate the presence of inflammation in psoriatic patients. In addition, sICAM-1 (OR 1.012 CI 1.003–1.022) was another marker significantly associated with psoriatic disease (Table 4). Raised levels of sICAM-1 reflect endothelial cell activation. After adjusting for the effects of age and gender, higher sP-selectin (OR 1.101 CI 1.005–1.228) levels were significantly associated with disease presence (Table 4). An elevated concentration of sP-selectin indicates platelet activation, as this adhesion molecule is expressed on the platelet surface upon activation.

TEG was performed on WB samples from healthy controls and psoriatic patients to assess coagulation sufficiency. Seven WB clot parameters were assessed in this study (Table 2) and the distribution of TEG parameters are illustrated by box-and-whisker plots in Fig. 2. Significant differences were detected in five TEG parameters (Fig. 2 and Table 5). Logistic regression modelling showed that shortened R (OR 0.682 CI 0.497–0.877) and K (OR 0.384 CI 0.169–0.733) values were significantly associated with psoriatic disease. Accelerated fibrin cross-linking, indicated by an increase in A (OR 1.157 CI 1.043–1.302), was also identified as a significant parameter. Moreover, after adjustment, an increase in the MRTG (OR 1.637 CI 1.046–2.894) was also significantly associated with psoriatic disease. Furthermore, a significant association was also detected between a shortened TMRTG (OR 0.778 CI 0.636–0.922) value and disease presence. Altogether, these results indicate that an altered coagulation profile, characterised by an increased tendency to form a blood clot, is associated with psoriatic disease.

The fibrin network architecture may influence clot properties; therefore, SEM was utilised to detect differences in the ultrastructure of fibrin fibre networks (PPP clots) from healthy individuals and psoriatic individuals. Representative scanning electron micrographs of PPP clots of a healthy control and psoriatic patients are depicted in Fig. 3A–D. Typically, a fibrin network from a healthy individual (shown in Fig. 3A) appeared as a 'loose' network of fibrin fibres. Individual fibres were clearly discernible, and pores were observed regularly between fibres. In contrast, fibrin networks of psoriatic individuals seemed to be more compact (Fig. 3B–D). Individual fibrin fibres could not always be distinguished, and multiple fibres were fused (indicated by the boxes in Fig. 3B, C).

Fibrin fibre diameter was measured in a subset of healthy individuals and psoriatic individuals. Psoriatic patients included in the subset were chosen to represent low, moderate, and high disease activity of PsA (according to DAPSA scores), with mild to moderate skin involvement (according to PASI scores), to ensure that disease activity of both the joint and skin domains were taken into account. The mean, minimum, and maximum fibre diameter were determined for each individual in both the control and patient groups. For the minimum and maximum fibre diameter, the smallest measurement and largest measurement of an individual were grouped as follows: minimum of healthy individuals, maximum of healthy individuals, minimum of psoriatic patients, and maximum of psoriatic patients. Figure 2 shows box-and-whisker plots illustrating the distribution of these variables in the study populations. Frequency bar graphs were constructed, as indicated in Fig. 4, to graphically illustrate differences in the distribution of fibrin fibre diameter in healthy individuals and psoriatic patients. In comparison to healthy controls, the fibrin fibre diameter was significantly increased in psoriatic individuals (Fig. 2). This was also reflected by the frequency bar graphs which show a greater count of thicker fibres in psoriatic patients (Fig. 4B). Logistic regression analysis showed that an increased fibrin fibre diameter (OR 1.562 CI 1.234–2.173) was a significant predictor of disease presence (Table 6). Similarly, an increase in the minimum (OR 1.483 CI 1.126–2.225) and maximum fibrin fibre diameter (OR 1.121 CI 1.041–1.240) were also associated with the presence of psoriatic disease (Table 6).

Discussion

Psoriasis is a T-cell mediated chronic, inflammatory skin disease characterised by the hyperproliferation of keratinocytes. However, several extracutaneous manifestations have also been linked with the condition[42,43]. In particular, an increased burden of cardiovascular morbidity and mortality has been observed in psoriatic individuals. Nevertheless, the causes responsible for CVD prevalence in psoriatic disease have not been fully elucidated. Thrombotic complications, such as MI[19,21], stroke[18,21] and VTE[8], have been shown to occur more frequently in psoriatic individuals. In the present study, we show that psoriatic patients presented with a biomarker profile that reflected systemic inflammation, endothelial activation, and heightened platelet activity. Moreover, altered viscoelastic properties of WB as well as structural changes in the fibrin network ultrastructure were observed in these individuals. See Fig. 5 for a summary of results.

The presence of a peripheral inflammatory milieu in psoriatic patients was confirmed by the association between increased levels of CRP and SAA and the condition (Table 4). These proteins are acute-phase reactants which are hepatically synthesised and released into circulation in response to inflammation and/or tissue injury. TNF-α, a major mediator of psoriasis pathogenesis, has been shown to induce the expression of IL-6. This cytokine, which is also produced by keratinocytes in psoriatic skin lesions[44] and the inflamed synovium in PsA[45], stimulates the synthesis of CRP. Therefore, elevated CRP levels may reflect the participation of pro-inflammatory cytokines in the psoriatic disease process. SAA is also overexpressed by epidermal keratinocytes in lesional skin of psoriatic patients[46] and may also be detected in the inflamed synovial tissue of PsA patients[47]. Both CRP and SAA possess procoagulant activity and may contribute to a hypercoagulable state via the induction of TF in endothelial cells and monocytes as well as the suppression of anticoagulant activity[48–52]. Furthermore, endothelial cell activation, indicated by raised sICAM-1 levels, was also associated, albeit weakly, with the presence of disease (Table 4). Membrane-bound ICAM-1 is involved in leukocyte extravasation to sites of inflammation[53], and the overexpression of this CAM has been demonstrated in lesional and non-lesional skin of psoriatic individuals[54]. ICAM-1 may promote thrombus formation by facilitating the adhesion of activated

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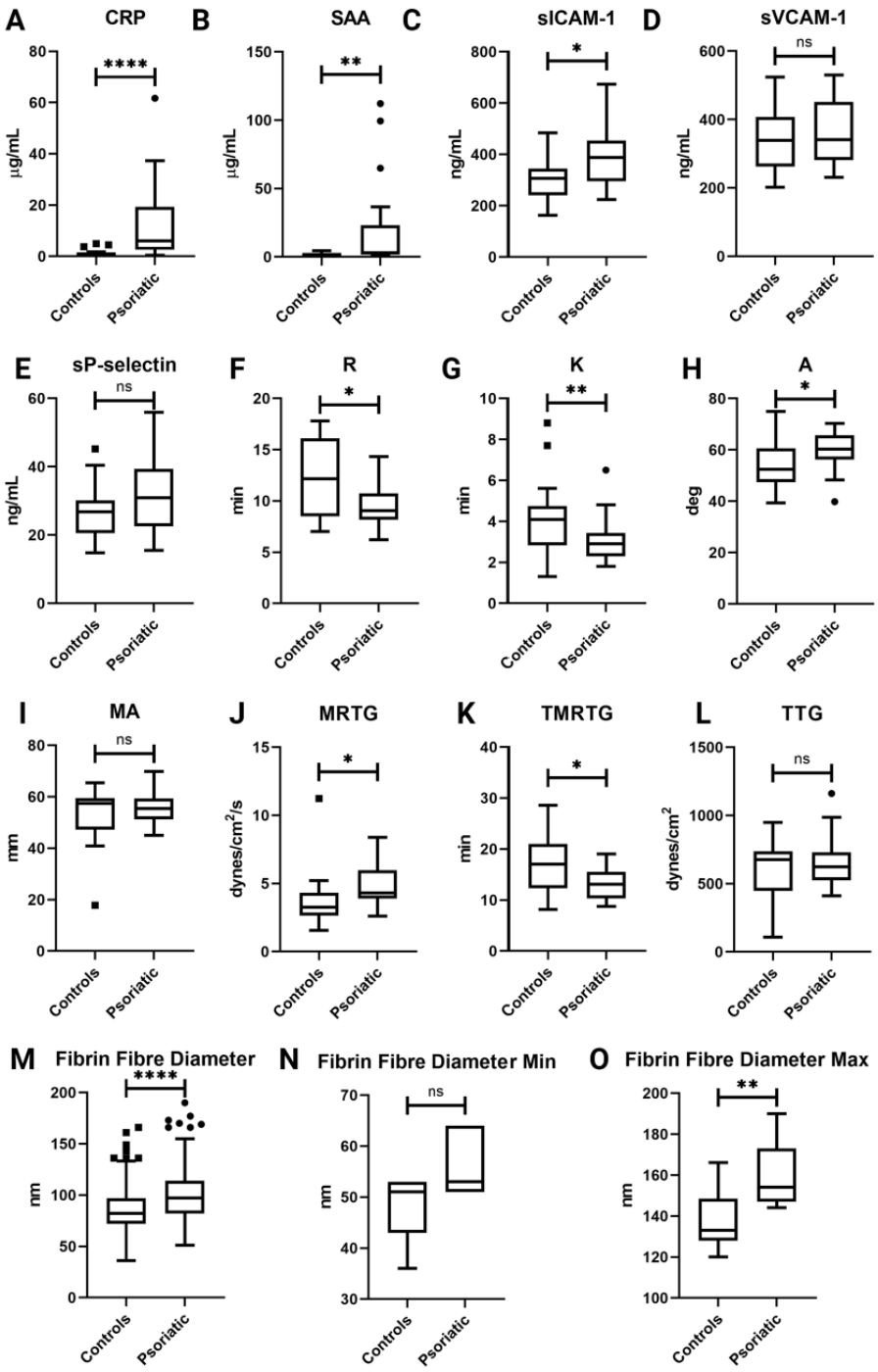

Figure 2. Box-and-whisker plots illustrating the distribution of parameters assessed in this study in healthy individuals and psoriatic patients. (**A**–**D**) indicates V-PLEX panel results, (E) indicates sSELP ELISA results, (F–L) indicates TEG results, and (**M**–**O**) indicates fibrin fibre diameter results. Asterisks indicate statistically significant differences between the groups, as determined by either an unpaired t-test or Mann–Whitney test, where ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$. Box-and-whisker plots were produced using GraphPad Prism version 8.4.3.

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Inflammatory markers	Healthy individuals (n = 20)	Psoriatic patients (n = 20)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
CRP (µg/mL)	0.57 (0.295–1.508)	6.015 (2.565–19.082)	1.375 (1.145–1.724)*	1.402 (1.146–1.838)*
SAA (µg/mL)	1.11 (0.59–2.615)	2.66 (2.02–15.332)	1.145 (1.034–1.320)*	1.144 (1.038–1.315)*
sICAM-1 (ng/mL)	306.15 (270.36–342.47)	388.48 (314.75–449.47)	1.009 (1.002–1.019)*	1.012 (1.003 –1.022)*
sVCAM-1 (ng/mL)	338.94 (279.42–400.59)	340.93 (297.68–449.79)	1.003 (0.996–1.011)	1.005 (0.997–1.014)
Platelet activation marker	**Healthy individuals (n = 15)**	**Psoriatic patients (n = 20)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
sP-selectin (ng/mL)	26.78 (22.825–29.34)	30.86 (23.873–39.013)	1.064 (0.987–1.159)	1.101 (1.005–1.228)*

Table 4. Results of logistic regression on laboratory parameters in healthy individuals and psoriatic patients. Data is expressed as median and (25–75% quartile range). ORs are reported at 95% CIs. Asterisks indicate statistically significant associations.

TEG WB clot parameter	Healthy individuals (n = 20)	Psoriatic patients (n = 20)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
R (min)	12.15 (8.7–15.975)	9.05 (8.25–10.575)	0.732 (0.555–0.919)*	0.682 (0.497–0.877)*
K (min)	4.095 (2.875–4.65)	2.9 (2.5–3.275)	0.490 (0.242–0.816)*	0.384 (0.169–0.733)*
A (deg)	52.45 (48.05–60.525)	60.25 (57.325–63.825)	1.112 (1.021–1.224)*	1.157 (1.043–1.302)*
MA (mm)	57.45 (49.125–58.9)	55.4 (52.25–59.4)	1.048 (0.976–1.145)	1.055 (0.969–1.178)
MRTG (dynes/cm²/s)	3.255 (2.758–4.303)	4.315 (3.918–5.963)	1.530 (0.993–2.537)	1.637 (1.046–2.894)*
TMRTG (min)	17.05 (12.397–20.94)	13.125 (10.397–15.52)	0.821 (0.677–0.954)*	0.778 (0.636–0.922)*
TTG (dynes/cm²)	676.95 (483.79–719.4)	623.84 (547.41–717.96)	1.002 (0.998–1.005)	1.002 (0.998–1.006)

Table 5. Results of logistic regression on TEG WB clot parameters in healthy individuals and psoriatic patients. Data is expressed as median and (25–75% quartile range). ORs are reported at 95% CIs. Asterisks indicate statistically significant associations.

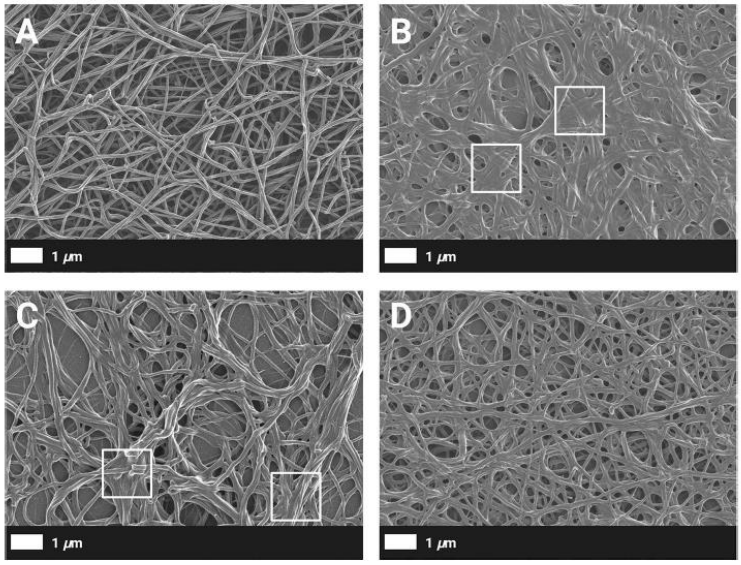

Figure 3. (A–D) Representative scanning electron micrographs of fibrin networks (prepared form PPP) from a healthy individual and psoriatic patients. (A) A typical fibrin network of a healthy 47-year-old female individual. (B–D) Fibrin networks of psoriatic individuals. (B) A fibrin network of a 45-year-old female individual with low PsA activity and mild skin involvement. (C) A fibrin network of a 40-year-old male individual with moderate PsA activity and mild skin involvement. (D) A fibrin network of a 49-year-old male individual with high PsA activity and moderate skin involvement.

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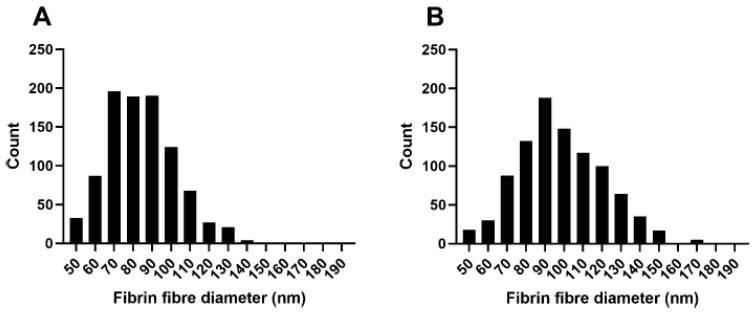

Figure 4. Frequency bar graphs of fibrin fibre diameter distribution. (**A**) indicates healthy individuals and (**B**) indicates psoriatic patients. Bar graphs were produced using GraphPad Prism version 8.4.3.

Fibrin fibre diameter	Healthy individuals (n = 9)	Psoriatic patients (n = 9)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Mean (nm)	83.724 (83.39–86.552)	99.043 (93.336–106.12)	1.470 (1.172–2.014)*	1.562 (1.234–2.173)*
Minimum mean (nm)	51 (46–53)	53 (51–62.25)	1.410 (1.107–2.062)*	1.483 (1.126–2.225)*
Maximum mean (nm)	133 (130–136)	160 (150–168.25)	1.112 (1.036–1.230)*	1.121 (1.041–1.240)*

Table 6. Results of logistic regression on fibrin fibre diameter in healthy individuals and psoriatic patients. Data is expressed as median and (25–75% quartile range). ORs are reported at 95% CIs. Asterisks indicate statistically significant differences.

platelets to the endothelium[55,56]. One may speculate that the increased plasma levels of the aforementioned molecules are due to a 'spillover' effect from affected skin areas, reinforcing peripheral inflammation.

Limited studies have investigated the coagulation profile of psoriatic individuals. We assessed blood coagulation using TEG, as it provides a more comprehensive view of coagulation status compared to conventional laboratory tests[57]. Shortened values of parameters that measure clot formation initiation and propagation (R, K) were associated with the presence of disease (Table 5). Enhanced clot propagation (A, MRTG, TMRTG) was also related to the presence of psoriatic disease (Table 5). These changes may be attributed to elevated levels of fibrinogen or an increased rate of thrombin generation. We did not measure fibrinogen levels in this study; however, other studies have reported raised levels in psoriatic patients[58–60]. Pro-inflammatory molecules, which circulate at elevated levels in active psoriasis, may induce TF expression by vascular endothelial cells and peripheral blood monocytes[61] while simultaneously suppressing the activity of thrombomodulin[62], leading to rapid thrombin generation and fibrin deposition. Despite an increased tendency to form a clot, parameters related to clot strength (MA, TTG) appeared unaltered in psoriatic individuals compared to healthy individuals (Table 5). This finding was likely caused by compromised fibrin network structure and/or platelet function in psoriatic patients. It should be noted that several patients that were included in this study received some form of immunosuppressant therapy (Table 3), which may be a confounder. Methotrexate and biological agents may potentially cause thrombocytopenia[63]. On the contrary, various studies have reported that platelets are in an activated state in psoriatic patients[64–66]. In the present study, platelets did appear to be activated as elevated sP-selectin levels were associated with psoriatic disease (Table 4). Importantly, refractoriness of platelets (as a result of hyperactivation) may decrease their ability to participate in the process of clot contraction, which may also influence the outcome of an occlusive thrombus. Tutwiler and colleagues (2017) have reported impaired clot contraction in acute ischaemic stroke patients, suggesting that this may lead to a greater reduction in intravascular blood flow[67]. A more recent study, by the same group, also notes that diminished clot contraction may be predictive of embolisation in pathological states[68].

Altered fibrin network architecture have been linked to the development of thrombotic disease[69], therefore, we assessed plasma clot ultrastructure with SEM. To our knowledge, this is the first study that has examined the fibrin network structure in psoriatic patients. In the current study, the fibrin network of psoriatic individuals appeared to be denser with various fused fibres (Fig. 3B–D). An increased fibrin fibre diameter (across all groups) was also more prevalent in psoriatic patients (Table 6). It has been reported that denser clots composed of thin fibrin fibres are more resistant to fibrinolytic degradation, than clots that are composed of thick fibres[31]. However, it should be kept in mind that various factors, such as the concentration of coagulation factors (*e.g.* thrombin[70]), cellular interactions (*e.g.* neutrophil extracellular trap formation[71]), and posttranslational modifications (*e.g.* oxidation[72]), may determine final clot structure. We hypothesise that the altered structural properties of fibrin clots along with an increased propensity to form a clot (as indicated by TEG results), may confer an increased risk for thrombosis in psoriatic patients. In addition, these patients may potentially be more susceptible to

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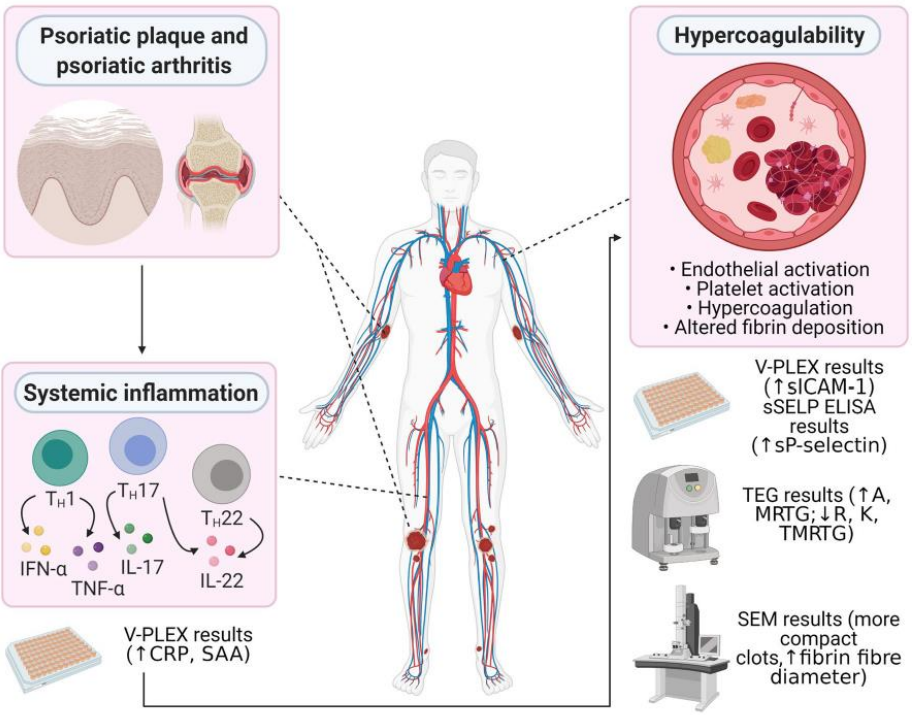

Figure 5. A summary of the key findings of this study. Various pro-inflammatory molecules are implicated in the onset and maintenance of psoriatic disease. These inflammatory mediators may spill over into circulation, resulting in systemic inflammation. Accordingly, psoriatic patients presented with elevated levels of acute-phase reactants (CRP and SAA). As the processes of inflammation and coagulation are interconnected, persistent systemic inflammation may promote the development of a prothrombotic state in psoriatic individuals. In this study, the prothrombotic state in psoriatic patients were characterised by endothelial (elevated sICAM-1 levels) and platelet activation (elevated sP-selectin levels), hypercoagulability (TEG results), and abnormal fibrin deposition (SEM analysis). Diagram created with BioRender.com.

embolisation, as platelet function seems to be impaired either due to hyperactivation, or a secondary effect of systemic therapy.

Our study has limitations. This study is cross-sectional in nature; therefore, no inferences can be made with regards to causality. In addition, the number of study participants was limited. Finally, psoriatic patients receiving systemic treatment were included in this study, which may have a potentially confounding effect. Further studies are required to confirm our findings.

Conclusion

Psoriatic disease is associated with an increased risk to develop CVD. Here, we confirm that circulating inflammatory molecules are significantly increased in our disease population. Our results therefore add to the accumulating evidence of the systemic inflammatory nature of psoriasis and the subsequent risk of cardiovascular comorbidities. We also show that psoriatic patients present with an altered coagulation profile, defined by an inclination towards thrombus formation. Additionally, we also demonstrate for the first time, that denser fibrin networks composed of thick fibrin fibres are formed in psoriatic individuals. These changes might have implications for the outcome of thromboembolic complications in the context of psoriatic disease. Future prospective studies should be conducted to confirm our findings and address remaining questions, such as determining the lytic susceptibility of an altered clot structure in psoriatic disease. We suggest that haemostatic function should be monitored carefully in psoriatic patients that present with severe disease and/or inflammatory flares, due to the pre-eminent risk of thrombotic complications. We recommend the routine monitoring of coagulability by a global coagulation assay, such as TEG, not as a predictor of imminent thrombotic risk, but rather as a preventative measure. Levels of fibrinogen and D-dimer may also be considered for this purpose, as they show strong prognostic value for the development of thrombotic events[73,74].

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Data availability

The datasets generated during and/or analysed during the current study are available in the Onedrive Blood Laboratory Repository at https://1drv.ms/u/s!AgoCOmY3bkKHiv9WfY1-yYCV8Bl5Iw?e=wYMG5N.

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Author contributions
M.J.E.V.: sample collection, preparation, and analysis, writing of paper, and preparation of figures. C.V.: technical assistance with SEM. T.J.R.: statistical analysis. G.T.: clinician. E.P.: study leader, corresponding author, funding. All authors reviewed and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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