AN INVESTIGATION OF LATE ONSET PSORIASIS

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy (PhD) in the Faculty of Medical and Human Sciences

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SCHOOL OF MEDICINE

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List of abbreviations

ACE	Angiotensin-converting enzyme
ACR	American College of Rheumatology
A+E	Accident and Emergency
AIT	Autoimmune thyroiditis
ANOVA	Analysis of variance
APC	Antigen presenting cell(s)
AS	Ankylosing Spondylitis
BDI	Beck depression inventory
BMI	Body mass index
BP	Blood pressure
CARD 14	Caspace-recruitment domain-containing protein 14
CCL	CC chemokine ligand
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1 gene
CI	Confidence interval
CLA	Cutaneous lymphocyte-associated antigen
cm	Centimetres
CSDN	Corneodesmosin
CTLA	Cytotoxic T-lymphocyte antigen
CXL	C-X-C motif chemokine ligand
DAB	Diaminobenzidine
DDX58	DEAD box polypeptide 58
DDC	Dermal dendritic cells
DLQI	Dermatology life quality index
DNA	Deoxyribonucleic acid
DSM-IV	Fourth edition of the diagnostic and statistical manual of mental disorders
EOP	Early onset psoriasis
ERAP1	Endoplasmic reticulum aminopeptidase 1
ET	Eleni Theodorakopoulou
Eth	Epidermal thickness
FoxP3	Forkhead box P3
GAD	Generalised anxiety disorder
GCP	Good clinical practice
GP	General practitioner
GPP	Generalised pustular psoriasis
GWAS	Genome-wide association study
HADS	Hospital anxiety and depression score
HADS-A	Anxiety subscale of the hospital anxiety and depression score
HADS-D	Depression subscale of the hospital anxiety and depression score
HBV	Hepatitis B virus
HCV	Hepatitis C virus

H&E	Haematoxylin and eosin
HIER	Heat induced epitope retrieval
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPA	Hypothalamic-pituitary-adrenal
HPF	High power fields
HRP	Horseradish peroxidase
HTN	Hypertension
iChip	Immunochip
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMCs	Intraepidermal collections of mononuclear cells
Ig	Immunoglobulin
IHC	Immunohistochemistry
IHD	Ischaemic heart disease
IFN-γ	Interferon gamma
IL	Interleukin
IL-36RN	Interleukin 36 receptor antagonist
IVL	Involucrin
JAK	Janus kinase
kg	Kilograms
KIR2DL	Killer-cell immunoglobulin-like receptor 2 DL-1
LC	Langerhans' cells
LCE	Late cornified envelop gene
LOP	Late onset psoriasis
LPP	Localised pustular psoriasis
LPS	Lipopolysaccharide
m	Metres
mm	Millimetres
µm	Micrometres
MHC	Major histocompatibility complex
MIF	Macrophage migration inhibitory factor
N or **n**	Total number
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHS	National Health Service
NK	Natural killer cells
NKT	Natural killer T-cells
NPF	National Psoriasis Foundation
NSAIDs	Non-steroidal anti-inflammatory drugs
OCT	Optimal cutting temperature
OR	Odds ratio
P	P value

p	French "petit"-short arm of a human chromosome
PAS	Periodic acid Schiff
PASI	Psoriasis area and severity index
PCT	Primary Care Trust
pDC	Plasmacytoid dendritic cells
PPD	Purified protein derivative
PDE4	Phosphodiesterase 4
PG	Prostaglandins
PGE_2	Prostaglandin E_2
PGPA	Patient global psoriasis assessment
PHAS	Psoriasis histological assessment score
PMN	Polymorphonuclear leukocytes
PLE	Polymorphic light eruption
PN	Uninvolved (non-lesional) skin from patients with psoriasis
PP	Involved skin (psoriasis plaque) from patients with psoriasis
PPP	Palmoplantar pustulosis
PRP	Pityriasis rubra pilaris
PsA	Psoriatic arthritis
PSORS	Psoriasis susceptibility gene
PSWQ	Penn State worry questionnaire
PTPN22	Protein tyrosine phosphatase, non-receptor type 22
PUVA	Psoralen plus ultraviolet light A
QoL	Quality of life
r	Pearson's Correlations Coefficient test
ρ	Spearman's rank Correlation Coefficient test
RA	Rheumatoid Arthritis
RANTES	Regulated on activation, normal T-cell expressed and secreted chemokine
RCT	Randomised controlled trial(s)
R&D	Research and development
RNA	Ribonucleic acid
RUNX3	Runt-related transcription factor 3
SAPHO	Synovitis, acne, pustulosis, hyperostosis and osteomyelitis syndrome
SCID	Severe combined immunodeficiency
SD	Standard deviation
SIR	Salford integrated records
SLE	Systemic lupus erythematosus
SLR	Single-lens reflex camera
SMA	Sympathetic-adrenomedulary
SNPs	Single nucleotide polymorphisms
SPAP	Salford psoriasis assessment proforma
SRFT	Salford Royal NHS Foundation Trust
STAT3	Signal transducer and activator of transcription 3

TAGAP	T-cell activation RhoGTPase activating protein
Th	T helper cells
TAU	Trauma assessment unit
TBS	Tris buffered saline
Tc	T cytotoxic cells
TCR	T-cell receptor
THS	Total histological score
TIS	Total inflammatory score
TMS	Total morphological score
T2DM	Type 2 Diabetes mellitus
TNF-α	Tumour necrosis factor alpha
Treg	T regulatory cells
URTI(s)	Upper respiratory tract infection(s)
UVA	Ultraviolet A
UVB	Ultraviolet B
UK	United Kingdom
UVR	Ultraviolet radiation
USA	United states of America
VAS	Visual analogue scale
VLA1	Very late antigen 1
vs	Versus
v/v	Volume per volume
w/v	Mass per volume
WHO	World Health Organisation
x^2	Chi square
y	Years
ZC3H12C	Zinc finger CCCH-type containing 12C
5-HTR2A	Serotonin 2A receptor

Abstract

This thesis entitled “An investigation of late onset psoriasis” was submitted by Eleni Theodorakopoulou to the University of Manchester, for the Degree of Doctor of Philosophy, in the Faculty of Medical and Human Sciences, on December 2013.

Psoriasis is a chronic, clinically heterogeneous, skin condition that affects approximately 2% of the general population. In 1985, Henseler and Christophers, classified psoriasis into early onset (EOP; age at onset ≤40 years-y) and late onset disease (LOP; age at onset >40 y). Previous research suggests that there are genetic and immunological differences between EOP and LOP. In particular, the major genetic determinant for psoriasis, the *human leukocyte antigen* (*HLA)-Cw6* allele, occurs more frequently in EOP (55-80%) compared to LOP (15-20%) patients. Epidermal Langerhans' cells (LC) migration is also different in these 2 subtypes of psoriasis. The primary aim of this thesis was to further explore the clinical, histological and immunohistochemical (IHC) differences between EOP and LOP. We compared clinical characteristics in a total of 497 subjects, including 340 psoriasis patients (108 recruited prospectively; 76 EOP and 32 LOP, mean age of onset 20.3±9.9 and 55.6±7y respectively, and 232 retrospectively; 202 EOP and 30 LOP, mean age of onset 20.7±9.9 and 55.2±7.2y respectively) and 157 controls (mean age 66±11.2y). Information on demographics, family history of psoriasis, clinical features, treatment and co-morbidities were recorded. Patients were also assessed for health-related quality of life and psychological distress. A total of 31 psoriasis patients, ≥ 50y of age, participated in the histological and IHC evaluation; 17 EOP and 14 LOP, mean age of onset 21.1±8.5 and 55.4±7.7y respectively. Skin biopsies were taken from involved (PP) and uninvolved (PN) skin and stained with haematoxylin and eosin (H&E) and IHC antibodies against various T-cell (CD3, CD4, and CD8) and LC (CD1α) markers. The H&E parameters (morphological and inflammatory) were graded with the use of a study specific histological score, whilst IHC positive epidermal cells were counted per microscopic field at 200X magnification. The dermal IHC infiltrate was assessed with a semi-quantitative (0-3) scale. Gender, body mass index, disease duration and severity, diagnosed hypertension and dyslipidemia were treated as covariates.

The clinical data showed that LOP patients had a lower likelihood of having a positive family history of psoriasis (62% of EOP versus 35.6% of LOP patients; *chi square-x^2, P=0.001*). In addition, patients with EOP parent(s) were 91% less likely to develop LOP than EOP *(odds ratio-OR=0.093, P=0.025, 95% confidence interval-CI 0.012-0.74*). Moreover, compared to LOP, EOP patients had a more severe disease (*x^2, P=0.021*), usually requiring 3rd line treatments *(x^2; P=0.010)*. They also experienced frequent flares, following upper respiratory tract infections (*x^2, P=0.049*). When data were segregated by age (≥50years) and after accounting for covariates, we observed that, compared to the non-psoriasis population, LOP patients were approximately 3 times more likely to develop type 2 Diabetes Mellitus (*OR=2.56, P=0.05, 95% CI 1.01-6.54*), whilst, EOP subjects were 98% less likely to develop autoimmune thyroiditis (*OR=0.025, P=0.02, 95% CI 0.001-0.55*). Psychologically, LOP patients were found to be a clinically more anxious group compared to EOP *(t-test, P=0.006*). Microscopically, the results from the H&E study showed an increased total inflammatory infiltrate in LOP, PP sections compared to EOP, PP ones (*t-test, P=0.028*). With IHC stains, we observed that in the epidermis of LOP PP, there was a significantly higher count of $CD4^+$ cells; mean $CD4^+$ in LOP of 15.1 ± 6.2 versus 6.7±4.6 in EOP (*Analysis of variance-ANOVA, P<0.001*). This subsequently led to a higher epidermal $CD4^+/CD8^+$ ratio of 1.3 in the LOP versus 0.5 for the EOP sections (*ANOVA, P=0.002*). In the PP dermis, $CD4^+$ were also more abundant in the LOP tissue (*x^2, P=0.049*). To assess whether these $CD4^+$ cells were either T-lymphocytes or LC, we examined for differences in the $CD3^+$ and $CD1\alpha^+$ cells. The mean epidermal $CD3^+$ tended to be higher in LOP PP sections; mean epidermal $CD3^+$ in the LOP 42.8 ± 13.3 versus 31.7 ± 17.5 in the EOP group (*ANOVA, P= 0.061*), while the dermal infiltrate showed a similar pattern (*x^2,P=0.067*). Finally, there was no difference in epidermal and dermal $CD1\alpha^+$ and $CD8^+$ cells in PP between EOP and LOP sections.

These data indicate differences in clinical phenotype, heritability, comorbidities and immunopathomechanism between EOP and LOP. Taken together they provide further evidence that EOP and LOP may be different diseases.

Declaration

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Dedication

I dedicate this thesis to my father; Frank Theodorakopoulos, who has taught me that perseverance and diligence holds the key to success. He kept me motivated along the way and pushed me to work hard for this PhD.

Published abstracts arising from this thesis

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1. Introduction

In this chapter, a literature review of the background of this thesis is presented. In the first part of the chapter, an overview of the clinical and genetic components, psychosocial impact, associated comorbidities and pathomechanisms of psoriasis is provided. Subsequently, a comprehensive discussion of the current classification schemes used to characterise psoriasis is outlined. A review of the literature, pertaining to early onset (EOP) and late onset psoriasis (LOP) follows, laying the foundations for the aims and objectives underlying this thesis.

1.1 Historical background of psoriasis

"Psoriasis is an antidote for dermatologists' ego"

Paul E. Bechet (reviewed in Bechet, 1936)

Psoriasis is a chronic, common, non-contagious, papulosquamous, skin disease of undefined aetiology. The history of psoriasis begins in ancient Greece, when "psoriasis" and "leprosy" were perceived and treated as the same disease. In those days, psoriasis patients suffered huge social rejection and stigma (Cowden and Voorhees, 2008).

For centuries, the clinical diagnosis of psoriasis remained ambiguous. It was not until the 19th century, when dermatologists attempted to establish a proper classification model to better understand and treat skin diseases, that psoriasis was distinguished from leprosy. Robert Willan, in his fundamental treatise on skin disease, "*On Cutaneous Diseases"*, was the first to describe psoriasis as a distinct clinical entity and place it in the group of papulosquamous skin diseases (reviewed in Griffiths and Barker, 2007b).

Recent advances in the fields of histo- and immunopathology, as well as molecular genetics have substantially improved our understanding of psoriasis, however, there are still numerous questions that remain unanswered (Cowden and Voorhees, 2008).

1.2 Epidemiology of psoriasis

Psoriasis is widely distributed throughout the world with a prevalence that ranges from 0.91 to 8.5% in adult patients and 0 to 2.1% in children (Parisi et al., 2012). General population-based surveys show a prevalence of approximately 2% amongst Caucasians (1.6% in the United Kingdom, 2.2% in the United states, 2.8% in the Faroe Islands, 2% in Sweden, 1.17-1.43% in Spain, 4.8% in Norway) and less than 1% in the Mongoloid race (Hong Kong, Japan and China); (Neimann et al., 2006; Gudjonsson and Elder, 2007). Overall there is no consensus on whether gender influences the prevalence of psoriasis but it is generally believed that it affects both males and females equally (Bovenschen et al., 2005). Additionally, there is literature showing a seasonal

distribution of the disease, with a higher frequency of flares or new cases reported in winter and spring (Farber and Peterson, 1961).

Despite multiple studies on the prevalence of psoriasis, relatively few have investigated its incidence (Setty et al., 2007; Icen et al., 2009a; Valdimarsson et al., 1986; Prinz, 1999; Vena et al., 2010). This is attributed to the substantial variation of the clinical phenotype of psoriasis and the absence of solid criteria for its diagnosis (Icen et al., 2009a). Although susceptible to limitations, studies on the incidence of psoriasis suggest an increase in the risk of developing the disease, in Western populations, between 1970 and 2011 (Setty Ar, 2007; Icen et al., 2009b; Gudjonsson and Elder, 2007; Parisi et al., 2013; Vena et al., 2010). It is, however, unclear whether the aforementioned observations on the incidence of psoriasis illustrate a true increase or are driven from a subsequent increase in risk factors for psoriasis, including stress and obesity, as well as increased awareness of the disease, in addition to more precise diagnostic methods and improved data collection strategies (Tollefson et al., 2010). Future research is, therefore, required to determine, more precisely, the incidence rate of psoriasis, while, at the same time, identify and control for all the relevant confounding factors, described above.

1.3 Clinical phenotypes of psoriasis

There is a large body of literature on the classification of psoriasis, from when Robert Willan published his classification until today. Despite psoriasis being a highly visible disorder, no specific diagnostic or predictive tests exist. From the pool of diagnostic strategies that exist in clinical practice, the most commonly used in dermatology to diagnose psoriasis has been clinical pattern recognition. Histological analysis of psoriasis plaques may sometimes be useful in helping with the differential diagnosis, but it is not a substitute for clinical examination nor it is routinely required (Naldi and Gambini, 2007). The common types of psoriasis are summarised in the International Psoriasis Council classification and consist of the following (Griffiths et al., 2007b):

1.3.1 Plaque psoriasis

Plaque psoriasis or psoriasis vulgaris represents the most prevalent clinical phenotype and affects 80% to 90% of patients with psoriasis (**Figure 1.1A**). The primary lesions include well-circumscribed, erythematous plaques with overlying silvery scale. These plaques have varying degrees of thickness (from thin ≤0.75 millimetres-mm to thick plaques >0.75 mm in elevation), size (from small ≤3 centimetres-cm to large plaques >3 cm in diameter) and distribution (usually located on the scalp, trunk and extensor surfaces of the elbows and knees); (Griffiths et al., 2007b).

There are various clinical signs that assist with the clinical diagnosis of plaque psoriasis. A blanched halo in the periphery of the plaques (*Woronoff's ring*) is often present; especially after phototherapy and coal tar treatment (Gupta et al., 1986); (**Figure 1.1B**). The pathogenesis of *Woronoff's sign* is not well understood, but one theory suggests that it may be driven by a decreased production of prostaglandins (PG) in the active edge of chronic phase psoriasis plaques (Penneys et al., 1976). Prostaglandins are lipid autacoids, derived from arachidonic acid, which can

induce vasodilation (prostaglandin E_2 or PGE_2) and hence promote the influx of inflammatory cells from peripheral blood to the skin. In addition, PGE_2 directly promotes the differentiation and maturation of certain inflammatory cells, which play a crucial role in the pathogenesis of psoriasis, the T helper (Th) 17 cells and are discussed later in this chapter (Boniface et al., 2009); (**chapter1**, **section 1.5.2**). Another clinical sign is scraping the scale off plaques, which results in punctuate bleeding and is known as *Auspitz's sign*. This is a result of the thinning of the epidermis, in combination with an increased endothelial cell proliferation and capillary ingrowth seen in psoriasis, which lead to a tortuous and fragile capillary network, very close to the surface of the skin. The *Koebner phenomenon* or "*isomorphic response*" describes the *de novo* appearance of plaques, upon injury of the skin and has been linked to severe, early onset, plaque psoriasis (Griffiths et al., 2007b). Few studies have investigated the incidence of *Koebner phenomenon* and results show a wide range in the frequency of occurrence*;* one study of 100 patients showed a 90% frequency of occurrence, while others demonstrated a 13% to 27% range of incidence (Camargo et al., 2013; Farber and Jacobs, 1974a; Gudjonsson et al., 2002; Kalayciyan et al., 2007). Post-inflammatory hypopigmentation of skin lesions, following resolution of plaque psoriasis, is known as *psoriatic leukoderma*.

The stage of development and severity of plaques can range from a few, scattered patches on the extensor surfaces (mild form of plaque psoriasis) to a severe, erythrodermic or pustular eruption, involving the whole body (severe form of plaque psoriasis). Nail changes are also frequent, especially in patients with joint disease, also known as psoriatic arthritis (PsA); (Peters et al., 2000); **Figure 1.2A**.

Based on the location of the plaques, psoriasis is divided into the following subtypes (localised forms of plaque psoriasis):

- *Flexural or intertriginous or inverse psoriasis* with thin plaques covered with minimal or no scale, located on the inframammary folds, axilla, intergluteal cleft, inguinal and genital region (Meier and Sheth, 2009); **Figure 1.3**. Skin fissures, accompanied by bleeding, pain and itching, are quite common. Napkin psoriasis is the term used to describe inverse psoriasis involving the diaper area in children under the age of 2 years.

- *Seborrhoeic-like psoriasis or sebopsoriasis* shares similar clinical and distributional features with seborrhoeic dermatitis. Young people are more frequently affected by this form of psoriasis. Sebopsoriasis has a predilection for sebum-rich areas of the body, such as scalp, eyebrows, nasolabial creases, ears, sternum and between the shoulder blades. Psoriatic plaques exhibit a very similar clinical presentation to seborrhoeic dermatitis, which is described as well-defined, thin, erythematous patches with dry, moist or greasy scale (Gupta and Bluhm, 2004). The presence of recalcitrant seborrhoeic dermatitis on the scalp which over the years progressed to classic scalp psoriasis, is common (Shemer et al., 2000).

- *Scalp psoriasis* is usually confined within the hairline region (**Figure 1.4**). In psoriasis, the scalp is usually the first part of the body which is affected, whilst 80% of plaque psoriasis patients have scalp involvement (Papp et al., 2007). The pre and post-auricular areas are

usually affected. The clinical picture is variable and comprises thick, bright red plaques, covered with thick scale to seborrhoeic plaques with scanty, fine scale.

- *Palmar/plantar psoriasis* is represented by symmetrically distributed red scaly plaques, within hyperkeratotic areas (*keratoderma*). Significant involvement of the nails is usually present. Weight-bearing areas of the soles and the centre of the palms are common areas of involvement (**Figures 1.2B**). Skin fissures of fingertips and heels are frequent. The differential diagnosis includes hyperkeratotic eczema and tinea pedis (Matsunaga et al., 1998).

- *Nail psoriasis;* nail manifestations are common in psoriasis, while 40-50% of patients have some degree of nail involvement (Griffiths and Barker, 2007b). These comprise both the nail matrix (pits, leukonychia, red spots in the lunula) and the nail bed (onycholysis, hyperkeratosis, salmon patches, splinter hemorrhage); (Farber and McClintock, 1968); **Figure 1.2A**. Recent research suggests that nail psoriasis may be a precursor of PsA (McGonagle, 2009; Williamson et al., 2004). The authors suggest that some histological features of nail psoriasis, such as enthesitis of the extensor tendon and involvement of the adjacent nail matrix, are implicated in the pathogenesis of PsA. Previous studies have reported a frequency of up to 80% of nail involvement in patients suffering from PsA, whilst severe nail psoriasis is associated with severe skin disease and deforming arthritis (Cassell et al., 2007).

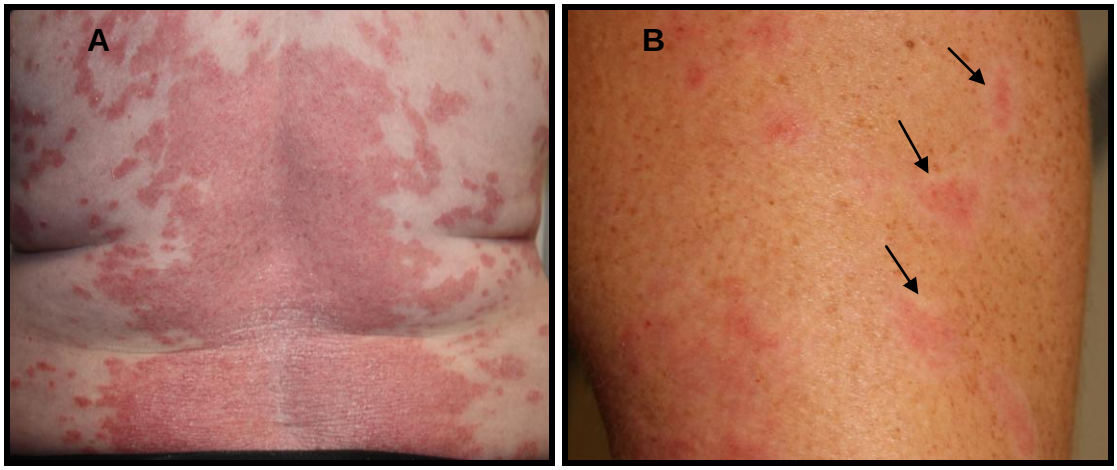

Figure 1.1 Chronic plaque psoriasis.

(A) Chronic plaque psoriasis on the back of a 60 years old, female subject, showing characteristic erythema, moderate thickness and moderate scaling. (B) Woronoff's sign shown on the right leg of a 30 years old, female patient; hypopigmented rings surrounding improving plaques (black arrows). Photographs were obtained from subjects of this study, with permission.

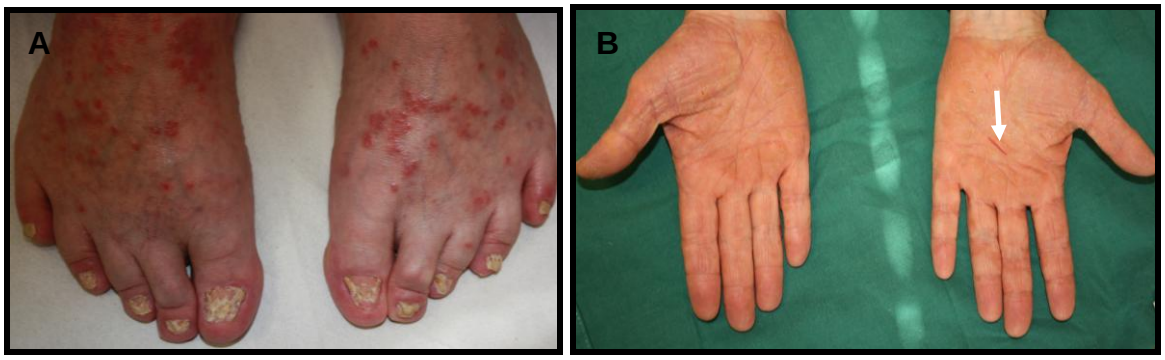

Figure 1.2 Nail and palmar psoriasis.

(A) Psoriasis affecting all ten toenails, with characteristic nail plate crumbling and subungual hyperkeratosis, in a 28 years old, male patient. (B) Psoriasis affecting both palms, with erythema, hyperkeratosis and scaling, from a 55 years old, female patient. Fissures are present in the centre of the left palm (white arrow). Photographs were obtained from subjects of this study, with permission.

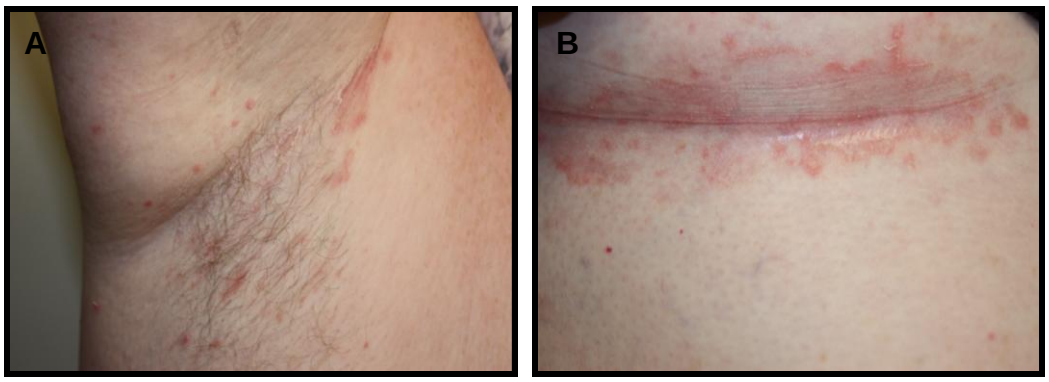

Figure 1.3 Intertriginous psoriasis.

(A) Inverse psoriasis on the right axilla, with mild erythema and fine scale. (B) Inverse psoriasis on the left inframammary crease. Photographs were obtained from a 60 years old female patient of this study, with permission.

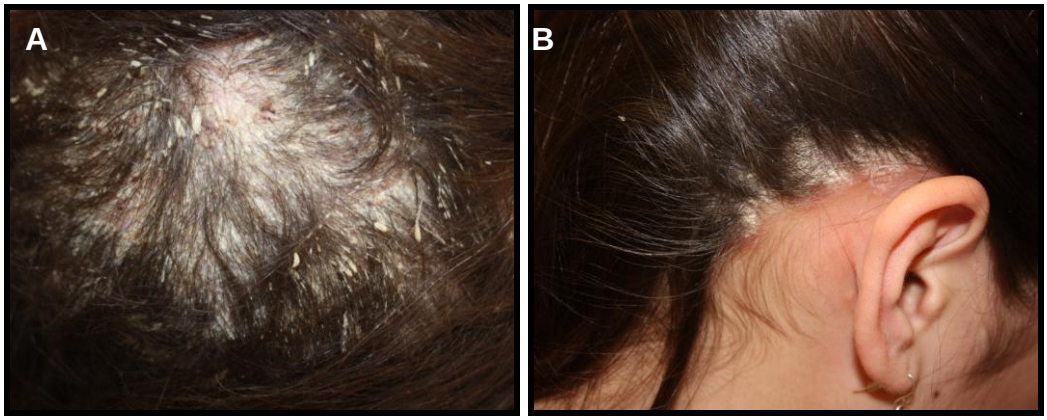

Figure 1.4 Scalp psoriasis.

(A) Scalp psoriasis on the vertex, with severe scaling and hair loss, from a 68 years old female patient. (B) Recalcitrant scalp psoriasis on multiple topical and systemic agents, from a 20 years old female patient. Photographs were taken from subjects of this study, with permission.

1.3.2 Guttate psoriasis

Guttate psoriasis; (guttate derives from the Latin word “gutta”=drop) is the second most common clinical phenotype of psoriasis; 2% of psoriasis patients are affected (Langley et al., 2005). It is recognized as the acute eruption of "teardrop" skin lesions (2-10 mm in diameter) of psoriasis and sporadic small plaques (<1cm in diameter). Guttate psoriasis usually affects the trunk, while the face and extremities can also be involved (**Figure 1.5**). There is a higher prevalence of the condition in children, teenagers and young adults, while it is associated with an antecedent throat infection or tonsillitis (Naldi et al., 2001). The microbe implicated in the pathogenesis of guttate psoriasis is the gram-positive bacterium, Streptococcus pyogenes (Telfer et al., 1992; Zhao et al., 2005). In addition, compared to other clinical forms of psoriasis, patients with guttate psoriasis are much more likely to be positive for the *human leukocyte antigen (HLA)-Cw06:02** allele (75-100% of cases); (Gudjonsson et al., 2002; Mallon et al., 1999; Tiilikainen et al., 1980). The prognosis of guttate psoriasis is typically good. The rash usually takes one month to develop, and if left untreated, usually resolves spontaneously during the third month. Sometimes guttate psoriasis progresses to chronic plaque. In addition, a sudden “guttate flare” occurring after streptococcal pharyngitis, is also seen in patients with established plaque psoriasis (Meier and Sheth, 2009).

Guttate psoriasis may sometimes mimic pityriasis rosea which is a common rash of small erythematous plaques with a fine scale at the borders of the lesion and with a distinct, Christmas tree-like distribution on the trunk (Meier and Sheth, 2009).

Guttate psoriasis may resemble lesions seen in secondary syphilis. The characteristic rash associated with secondary syphilis is a maculopapular eruption with fine scaling, usually affecting palms and soles (Browning, 2009). It can spread to involve the whole body. A skin biopsy, a rapid plasma reagin and a fluorescent treponemal antibody test can significantly help with diagnosis.

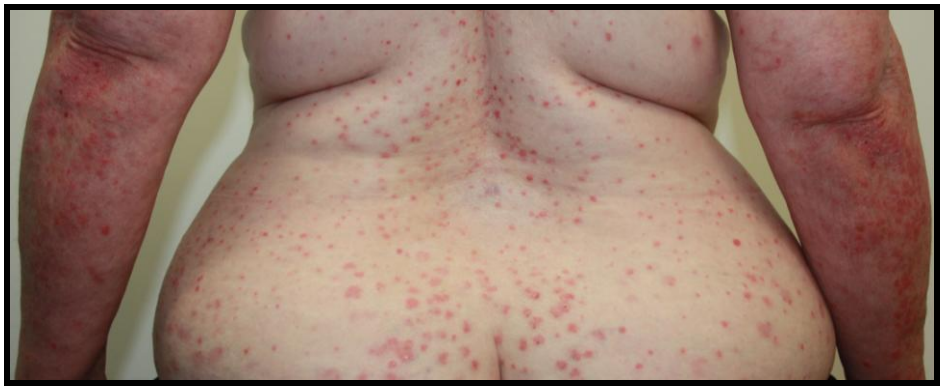

Figure 1.5 Guttate psoriasis.

Guttate psoriasis, also known as "eruptive" psoriasis, presents as small, erythematous, drop-like plaques. This figure shows the eruption of guttate psoriasis on the back of a 34 years old, female subject. Photographs were obtained from subjects of this study, with permission.

1.3.3 Pustular psoriasis

The term "pustular psoriasis" is used to describe skin conditions, with extensive development of pustules on the skin's surface (Amin and Maibach, 1998).

There are two types of pustular psoriasis: generalised pustular psoriasis (GPP) and localised pustular psoriasis (LPP).

1.3.3.1 Generalised pustular psoriasis

Generalised pustular psoriasis is a rare form of psoriasis which erupts as an acute, subacute or, rarely, progressive generalised skin rash of sterile pustules, on a background of erythematous, sore and inflamed skin. Nail changes, such as subungual pustules, are frequently present. Current research considers GPP as a separate entity from plaque psoriasis, which may develop de novo and resolve spontaneously or evolve into plaque psoriasis (Marrakchi et al., 2011).

There are various triggering factors that have been implicated in precipitating GPP; the most common being the sudden withdrawal from topical, high potency or systemic corticosteroids (Baker and Ryan, 1968). In addition, several medications have been reported to provoke GPP. These are non-steroidal anti-inflammatory drugs-NSAIDs (oxyphenbutazone, phenylbutazone), antibiotics (penicillin), lithium, morphine, anti-malarials (hydroxychloroquine), beta-blockers (propranolol), topical coal tar and salicylates (Lyons, 1987). Other reported triggers include upper respiratory tract infections (URTIs), exposure to ultraviolet radiation (UVR) and pregnancy (Baker and Ryan, 1968). Patients with GPP may experience systemic organ involvement, such as polyarthritis, liver dysfunction (cholestasis from neutrophilic cholangitis), renal impairment (acute tubular necrosis) and cardiac problems (Viguier et al., 2004; Warren et al., 1974).

1.3.3.2 Localised pustular psoriasis

Localised pustular psoriasis is divided in two subtypes: Acrodermatitis continua of Hallopeau and PPP.

- *Acrodermatitis continua of Hallopeau* was first described by Radcliffe-Crocker, in 1888, under the term dermatitis repens and was first linked to psoriasis by Barber (reviewed in Barber and Eyre, 1927). It is a chronic condition which usually develops after local skin injury of a single finger or toe. Tiny pustules arise at the site of trauma and sometimes, as a psoriasiform rash involving the dorsum of hands or feet. Nails are usually affected with onychodystrophy and sometimes, even anonychia.

- *Palmoplantar pustulosis (PPP)* predominates in older age groups (5^{th} to 6^{th} decade of life) and affects mainly females. The clinical picture of PPP includes hyperkeratosis and collections of pustules on the palms and soles (**Figure 1.6**). Extracutaneous manifestations, such as arthro-osteitis and thyroid disease, have been described in the literature (Edlund et al., 1988). Palmoplantar pustulosis is also associated with the SAPHO (synovitis, acne, pustulosis, hyperostosis and osteomyelitis) syndrome (Schilling, 2004). Although PPP can coexist with plaque psoriasis in 20% of cases, it does not share the same genetic, epidemiological and clinical characteristics as psoriasis (Griffiths et al., 2007b; Brunasso et al., 2013). Therefore, it is now considered as an individual entity, separate from psoriasis (Asumalahti et al., 2003). Recent research suggests that smoking, as well as the use of new biologic agents such as those inhibiting anti-tumour necrosis factor alpha (TNF-α), have been linked to the onset of PPP (O'Doherty and MacIntyre, 1985; Mössner et al., 2008).

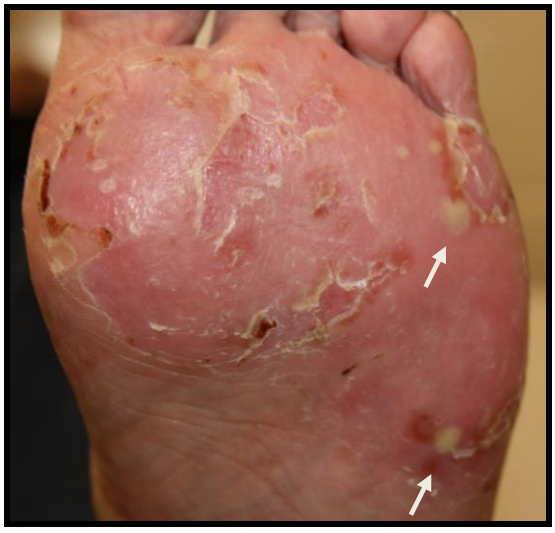

Figure 1.6 Palmoplantar pustulosis.

Palmoplantar pustulosis involving the left sole of a 51 years old, female subject. Characteristic visible collections of sterile pus are noted beneath the 5^{th} toe (white arrow). Photographs were obtained from subjects of this study, with permission.

1.3.4 Erythrodermic psoriasis

Erythrodermic psoriasis, a term first introduced by von Hebra in 1868, is a severe condition with widespread erythema, variable skin shedding, involving more than 90% of the body surface and sometimes is accompanied by serious metabolic dysfunction (Boyd and Menter, 1989; Misha et al., 2010; Rosenbach et al., 2010); (**Figure 1.7**). This is a severe type of psoriasis which requires hospitalisation and careful monitoring. It usually develops on a background of extensive plaque psoriasis. *De novo* erythrodermic psoriasis has also been reported. Common mediators of erythrodermic psoriasis are infections, lithium intake and sudden withdrawal from oral steroids (Rym et al., 2005).

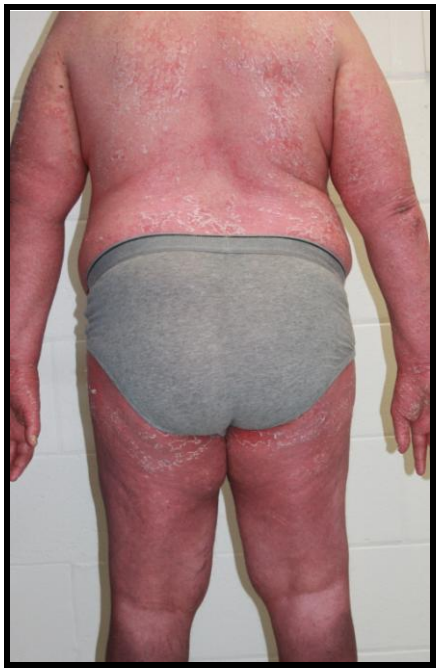

Figure 1.7 Erythrodermic psoriasis.

The figure shows a 33 years old, male patient affected with erythrodermic psoriasis, with the representative widespread erythroderma and exfoliation. Photographs were obtained from subjects of this study, with permission.

1.3.5 Eczematous psoriasis

Eczema is used to describe a range of skin diseases that present with erythema, skin oedema and dryness, in combination with pruritus. Infection of the skin, along with oozing and cracking of the skin are quite common.

The distinction between psoriasis and eczema can be difficult, leading some clinicians to use the term eczematous psoriasis. Eczematous psoriasis can be primary, where eczema is part of the clinical spectrum of psoriasis manifestations, or secondary, where an eczematous response to an exogenous irritant or allergen, affects the already established plaque psoriasis (Roenigk et al., 1998).

1.3.6 Photosensitive psoriasis

Photosensitive psoriasis is a phenotypically distinct subset of psoriasis. Exposure to sunlight provokes a photo-distributed psoriasiform rash. Ultraviolet A (UVA) radiation is considered as the key mediator.

Previous studies have linked photosensitive psoriasis to other photosensitive disorders such as polymorphic light eruption (PLE) and chronic actinic dermatitis (Ros and Wennesten, 1998). Recent work has demonstrated that this subset of psoriasis is characterised by a female predisposition, a positive family history and early onset of the disease (Rutter et al., 2009). Fair skin of phototype I or II seems to be a major risk factor.

1.4 Histology of normal skin

The skin is the largest organ of the human body and is readily visible. It has a protective and barrier function role against heat, sunlight, trauma and infections. It consists of three layers (Hunter, 1973); the epidermis (the outermost, avascular layer of the skin), the dermis and subcutis (subcutaneous layer or hypodermis); (**Figure 1.8A**). Keratinocytes are the main cell types of the epidermis with a normal turnover time of approximately 30 days. The keratinocytes are synthesized at the base of the epidermis and upon maturation they travel upwards and give rise to five epidermal sub-layers: stratum basale (the bottom sub-layer); stratum spinosum; stratum granulosum; stratum lucidum; stratum corneum (the outermost sub-layer of the epidermis); **Figure 1.9**. Apart from keratinocytes, various types of cells reside in the epidermis. Epidermal Langerhans' cells (LC) , serve as antigen-presenting cells (APC) and are involved in immune surveillance of the skin. Melanocytes reside in the stratum basale and produce melanin(eumelanin and pheomelanin), which is an absorptive pigment that protects the deoxyribonucleic acid (DNA) of keratinocytes from harmful UVR. In addition to keratinocytes and melanocytes, Merkel cells are found in the stratum basale. These are somatosensory receptor cells which are thought to play a key role in sensing the surface texture and shape of objects, with light touch.

The dermis lies between the epidermis and the subcutis; **Figure 1.8A**. It consists of collagen and elastic fibres and is composed of two zones, the papillary and reticular dermis. An extensive microvascular system resides in the dermis which is responsible for the nutrition and thermoregulation of the skin. Through the vascular system of the dermis, the cells of the immune system reach the epidermis.

Cells of the skin's immune system reside in both the epidermis and the dermis. These include dermal dendritic cells (DDC), macrophages, T-and B-lymphocytes, neutrophils, mast cells and eosinophils. Their roles will be discussed in more detail in **section 1.5.2 (Chapter 1)**. Smooth muscle endothelial cells line the lumen of the vasculature of the skin. Fibroblasts synthesize extracellular matrix material, which is a net-like structure consisting from fibre proteins (collagen, elastin) and glycosaminoglycans (hyaluronic acid, heparin sulfate and chondroitin sulfate).

Beneath the dermis lies the subcutaneous layer, which comprises adipocytes, fibroblasts and macrophages.

1.4.1 Histology of psoriasis

Histological analysis of psoriasis shows rapid turn-over of the epidermis as a result of accelerated proliferation and differentiation of epidermal keratinocytes (McKay and Leigh, 1995). In psoriasis, all phases of the cell cycle are shortened to such an extent that the total cell cycle lasts only 1.5 days, compared to the normal 30 days life cycle (Weinstein et al., 1985).

Histological sections of psoriasis, stained with haematoxylin and eosin (H&E), show a characteristic psoriasiform pattern; abnormal hyperproliferation and differentiation of keratinocytes, resulting in diffuse hyperplasia (acanthosis) of the Malpighian layer (stratum basale and stratum spinosum), with elongation of the rete ridges (downward projections of the epidermis which interlock with the papillary dermis), thickening of the cornified epidermal layer, atypical presence of nucleus-containing keratinocytes in the top layer of the epidermis (parakeratosis), absence of the granular layer (hypogranulosis) and increased dermal angiogenesis (Bovenschen et al., 2005); **Figures 1.8B** and **1.10C**. Increased mitosis in all cells of the basal layer as well as in suprabasal layers is prominent (Krengel et al., 1998). Widening of the spaces between keratinocytes (intercellular oedema) is seen in severe or early-stage psoriasis plaques (Beek and Reede, 1977). An abnormal skin barrier function following modification of the tight junction structure is present (Ghadially et al., 1996). Additionally, the expression of hyperproliferation markers such as keratins 6 and 16 in the suprabasal layer of the epidermis is found in both involved (PP) and uninvolved (PN) skin, compared to normal skin (Leigh et al., 1995; Bhawan et al., 2004).

Finally, a series of abnormal events take place in the dermal microvasculature. These include increased endothelial cell proliferation and neovascularisation resulting in the dermal papillary vessels appearing dilated, elongated and tortuous, with increased blood flow and permeability. These microvascular changes are the first to occur prior to any clinical evidence of epidermal hyperplasia and tend to disappear with disease clearance (Krengel et al., 1998).

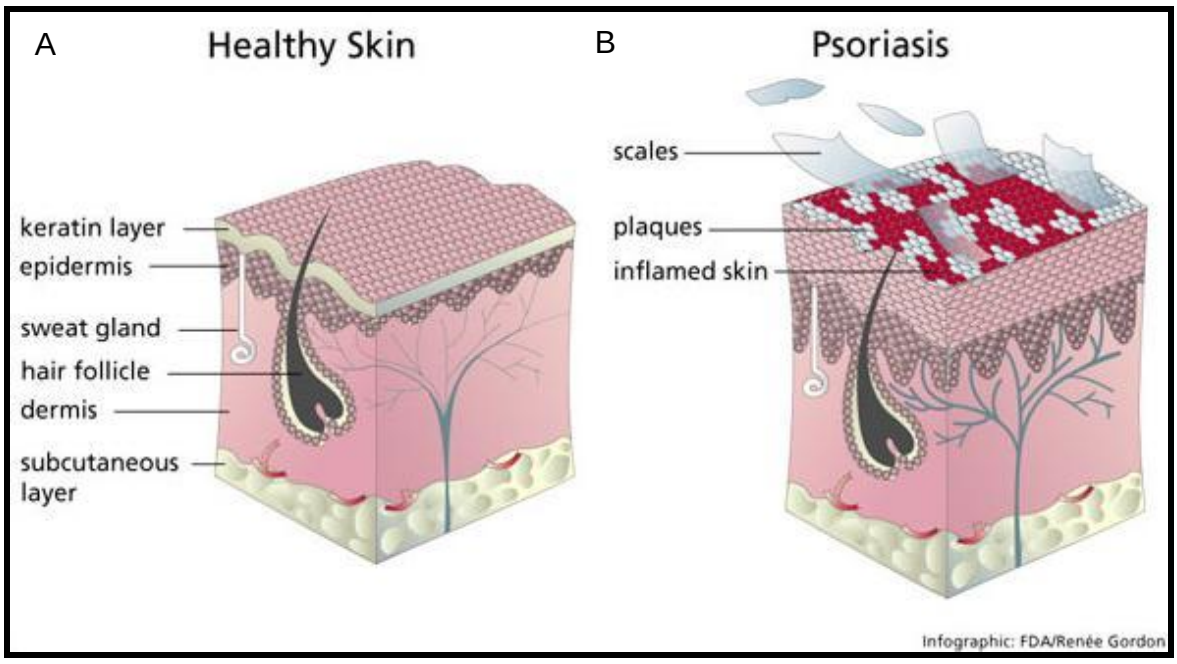

Figure 1.8 Comparison of the structure of normal skin and involved (PP) skin.

Normal skin (A) consists of three layers; the epidermis, the dermis and the subcutaneous layer. The epidermis consists of keratinocytes which form different layers. The outermost sub-layer of the epidermis (stratum corneum or keratin layer) consists of dead keratin-filled keratinocytes which shed daily. The thickness of normal skin depends on the body location and varies from 0.5 mm to 1.5mm. The dermis is between the epidermis and subcutaneous layer and consists of the papillary and reticular dermis. The dermis is also vascular with a complex capillary matrix. The dermis is connected to the epidermis with a fine net called dermo-epidermal junction, which has nutritive and absorption properties. Sweat glands and hair-follicles, which reside in the dermis, function to control thermoregulation. In psoriasis (B) the skin undergoes structural changes including thickening of the epidermis and dermis, as well as intense shedding. The microvasculature of the dermis becomes dilated and tortuous, which accounts for the characteristic erythema of PP. Figure obtained and reproduced from FDA/Renee Gordon, HHS.

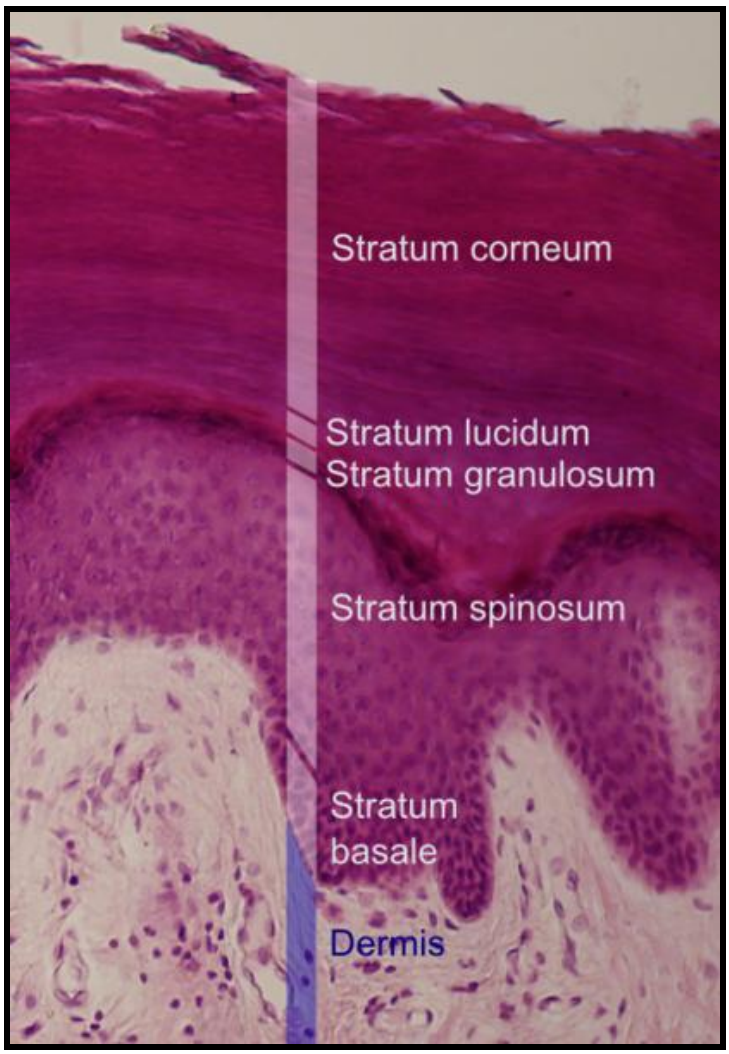

Figure 1.9 The sub-layers of the epidermis.

This figure shows the 5 sub-layers of the epidermis from the inner to the outer; stratum basale or germinativum is the inner sub layer of the epidermis, which comprises proliferating keratinocytes and melanocytes. Keratinocytes are attached to the dermo-epidermal zone via hemidesmosomes. In stratum spinosum, keratinocytes bind each other with desmosomes and form keratinosomes (lamellar bodies) which are important in the skin barrier function. Epidermal Langerhans cells (LC) reside in this layer. The stratum granulosum comprises anucleated keratinocytes which release keratinosomes to form a water resistant lipid barrier. The stratum lucidum or the "clear" layer is only found in anatomic sites with increased epidermal thickness (palms, soles) and consists of 3-5 layers of keratinocytes undergoing apoptosis. The stratum corneum consists of 10-30 layers of anucleated, acytoplasmic keratinocytes which bind together by corneodesmosomes and form a water-resistant, tight barrier. Figure obtained and adapted from doi: http://training.seer.cancer.gov/melanoma/anatomy/.

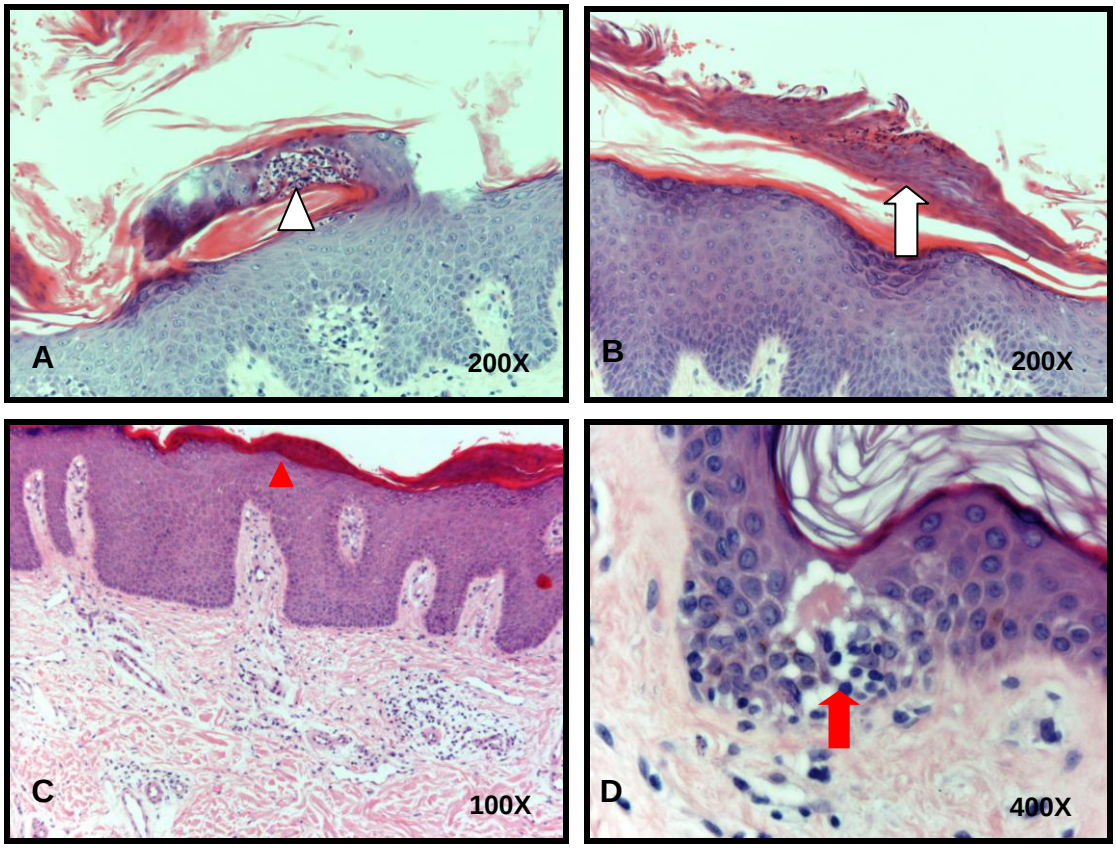

Figure 1.10 Histopathological changes seen in haematoxylin and eosin (H&E) sections of psoriasis skin.

Microimages of H&E sections from psoriasis plaques of subjects of this study and permission has been obtained; ***(A)*** *at 200X magnification, the white arrowhead shows a Kogoj spongiform pustule which consist of subcorneal collections of neutrophils,* ***(B)*** *Munro microabscesses are seen in the stratum corneum and comprise collections of neutrophils (arrow, white),* ***(C)*** *at 100X magnification, acanthosis of the epidermis. Absent stratum granulosum (arrowhead, red). Prominent vascular changes, as well as dense a lymphocytic infiltrate are also apparent in the dermis,* ***(D)*** *Non-lymphoid intraepidermal collections of mononuclear cells or pseudo-Pautrier abscesses in uninvolved skin which is adjacent to a psoriasis plaque (arrow, red), seen at 400X magnification.*

1.4.2 Inflammatory changes of psoriasis

Under H&E staining, the microscopic presence of neutrophilic collections in the stratum corneum (Munro microabscesses) and/or the subcorneal area (Kogoj spongiform pustules) is a characteristic feature of psoriasis (Du Vivier, 2002; Schon and Boehncke, 2005; Lowes et al., 2007); **Figures 1.10A** and **1.10B**.

A dense lymphocytic infiltrate is present in the epidermis and dermis. Antigen-presenting cells, including epidermal LC, plasmacytoid dendritic cells (pDC) and DDC are also abundant in psoriasis lesions. Non-lymphoid intraepidermal collections of mononuclear cells (ICMC; LC microgranulomas or pseudo-Pautrier abscesses) are sometimes seen underneath the stratum

corneum, usually in severe psoriasis cases (Krengel et al., 1998; Wolberink et al., 2011; Candiago et al., 2000); **Figure 1.10D**.

Occasionally, either a localised or a diffuse eosinophilic infiltrate of the dermis is also present. A prominent dermal eosinophilic infiltration in a background of psoriasiform dermatitis is a marker of drug-induced psoriasiform eruptions. Extensive collections of eosinophils in the epidermis are mainly seen in vesiculobullous conditions, including incontinentia pigmenti, bullous pemphigoid, pemphigus vulgaris and foliaceous and sometimes in allergic contact dermatitis. Only a limited number of reports exist on the functional role of eosinophils in non-drug-induced psoriasis (Lundin et al., 1990; Mansur et al., 2008; Schopf et al., 1998). The authors suggest that eosinophils can display immunomodulatory properties and thus influence the inflammatory process in both the epidermis and the dermis of PP skin (Lundin et al., 1990).

The presence of mast cells in the papillary dermis, close to the dermo-epidermal junction is commonly seen in psoriasis (Harvima, Nilsson et al. 2008). Mast cells have been shown to play an important role in the inflammatory skin manifestations of early PP lesions (Toruniowa and Jablońska, 1988).

1.5 Pathogenesis of psoriasis

The aetiology of psoriasis is multifactorial. Its pathogenesis was thought to be primarily epidermal, caused by an uncontrolled mitosis of keratinocytes (Farber and Cox, 1967). However, the serendipitous finding of an immunosuppressant medication called cyclosporine, which demonstrated significant efficacy in treating psoriasis, gave an insight into the immunopathogenic nature of psoriasis (Mueller and Hermann, 1979). There is now increasing evidence that psoriasis is a result of a complex interaction between impaired barrier function and innate and adaptive immune systems, which is impacted by environmental and genetic contributions (Bovenschen et al., 2005; Krueger and Bowcock, 2005).

1.5.1 Genetics of psoriasis

There is strong evidence showing genetic predisposition leading to the development of psoriasis (Traupe, 1995). Twin studies have been important in studying its genetic and environmental causal factors. A twin study from the United States of America (USA) showed a concordance rate of 72% for identical twin pairs and 22% for dizygotes which strongly supports the genetic background of the disease (Farber and Nall, 1974b). An Australian study demonstrated a concordance rate of only 35 % in identical twins versus (vs) 12% in non-identical twins (Duffy et al., 1993). This phenotypic discordance in the identical monozygotic twins with psoriasis implies that the psoriasis phenotype is the interplay of genetic and environmental stimuli.

A positive family history predisposes to a higher disease incidence. Watson and colleagues demonstrated a risk of 50% when both parents were affected with psoriasis, 16% when either parent had the disease and 8% when there was no family history of psoriasis (Watson et al., 1972). Henseler reported a risk of 14% for developing psoriasis when either parents were affected

(Henseler, 1998). A clinical review of 1,262 psoriasis patients revealed that 71% of those with childhood psoriasis had a positive family history for the disease (Morris et al., 2001). Many studies on pedigree charts analysis of families with psoriasis have been carried out, investigating the effect of parental gender on the expression of the disease in the offspring (Burden et al., 1998; Lomholt, 1963; Swanbeck et al., 1994). Lomholt, to assess the prevalence of psoriasis, used census-based data from 10,000 inhabitants of the Faroe Islands and highlighted the presence of a multifactorial inheritance mode involving a polygenic-environmental interaction (Lomholt, 1963; Iselius and Williams, 1984). An autosomal dominant inheritance, with reduced penetrance has been suggested by other authors (Ananthakrishnan et al., 1974; Campalani and Barker, 2005). A study on the mode of inheritance of psoriasis in 5,197 Swedish families, revealed a single recessive gene with a frequency of 25% (Swanbeck et al., 1994). In Scotland, the possibility of genetic anticipation was explored in 764 psoriasis probands (Burden et al., 1998). The authors showed that psoriasis developed earlier in life in each successive generation, especially in offspring coming from affected fathers, compared to probands who inherited the disease from affected mothers.

Technological advances in genomics have led to many new genetic loci linked to psoriasis. The most consistently reported genetic region with the highest association is the *psoriasis susceptibility gene 1 (PSORS1);* (Deguchi et al., 2001; Nickoloff et al., 2000). *Psoriasis susceptibility gene 1* includes the major histocompatibility complex (MHC) region incorporating the HLA system (Prinz et al., 1994) and is located on the short arm (p) of chromosome 6 (Campalani and Barker, 2005; Tiilikainen et al., 1980). Fine mapping of *PSORS1* revealed that *HLA-Cw*06:02* is the main susceptibility loci for psoriasis, particularly in Caucasian populations (Cluster 17 Collaboration, 2005; Christophers, 2003; Szczerkowska-Dobosz et al., 2004; Nickoloff et al., 2000; Zhang et al., 2003). Mallbris and coworkers demonstrated an increased prevalence of positive streptococcal throat swabs in *HLA-Cw*06:02* positive patients, which may imply a functional interaction between *HLA-Cw*06:02* and streptococcal epitopes in the development of psoriasis (Mallbris et al., 2009). There is now evidence that *HLA-Cw*06:02*-encoding products may play a direct biological role in the pathogenesis of psoriasis by presenting peptide epitopes to $CD8^+$ cells (Johnston et al., 2004).This is also supported by the fact that $CD8^+$-MHC I interactions are very important for the functionality of $CD8^+$ (Onuma, 1994; Griffiths and Voorhees, 1992). In line with the previous, in patients with psoriasis flares preceding streptococcal pharyngitis, the presence of the same clonal T cell receptor (TCR) rearrangements in skin-infiltrating and tonsilar T-cells suggests that the T-cell epitopes shared between streptococci and the skin are identified by these skin-homing T-cells as self-antigens (Diluvio et al., 2006; Elder et al., 2010; Gudjonsson et al., 2004). These data indicate that psoriasis is primarily an immune-mediated disease.

*Human leukocyte antigen-Cw*06:02* is in strong linkage disequilibrium with *HLA-B* alleles (-*B13, Bw17, -Bw37*) and these are thereafter associated with psoriasis (Russell et al., 1972; Nickoloff and Wrone-Smith, 1999). However, the association of *HLA-B* alleles with psoriasis is not only secondary to a strong link of the disease with *HLA-Cw*06:02* but also directly associated with *the HLA-DR7* antigen (MHC II system) on B-cells (Prinz et al., 1994). Thus, both Class I and II MHC alleles play an important role in the pathogenesis of psoriasis. Recent genetic functional studies revealed the presence of *HLA-Cw*06:02* gene expression patterns, which are responsible for the regulation and expression of this risk allele in normal, PP and PN skin. In particular, the

authors identified two promoter single nucleotide polymorphisms-SNPs (*rs2524094* and *rs10657191)*, unique to *HLA-Cw*06:02,* which alter the function of *HLA-C* cytokines (Hundhausen et al., 2012). These results shed light on the role of *HLA-Cw*06:02* in the pathogenesis of psoriasis.

*Human leukocyte antigen-Cw*06:02* positive individuals have only a 10% risk of developing psoriasis, which means that the familial aggregation seen in psoriasis in not solely associated with *PSORS1* (Gudjonsson and Elder, 2007). The above suggests that additional non-MHC psoriasis susceptibility genetic loci exist. A study from Germany (Prinz et al., 1994) highlighted the strong link between *HLA-Cw*06:02* and the corneodesmosin (*CSDN*) gene, suggesting abnormalities in adhesion of keratinocytes. Recent genetic research has identified independently replicating non-MHC loci that are linked to psoriasis, as well as other diseases, such as Crohn's disease, type 2 Diabetes Mellitus (T2DM), Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA) and Ankylosing Spondylitis (AS); (Heath and Carbone, 2001; Smith et al., 2008b). These genes include the CDK5 regulatory subunit associated protein 1-like 1 gene (*CDKAL1*) (Heath and Carbone, 2001) and the protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*); (Smith et al., 2008b).

During recent years, considerably more attention has been paid to genes encoding regulatory cytokines. It has since become apparent that polymorphisms in these genes and subsequent identification of variant cytokine alleles can indicate susceptibility to certain immune-mediated diseases (Reich et al., 2002). Such genetic polymorphisms, linked to psoriasis, have been found on genes encoding the TNF-α promoter (Arias et al., 1997; Reich et al., 2002; Mossner et al., 2004), interleukin (IL)-12B and IL-23R; (Cargill et al., 2007). In addition, work by Smith and co-workers on the *PTPN22* gene links psoriasis to altered development of regulatory T-cells (Smith et al., 2008b). Taken together, the pathogenetic concept of psoriasis presumably lies in a T-cell-mediated autoimmune mechanism. Recently, a genome-wide association study (GWAS) carried out on a European population (Genetic Analysis of Psoriasis Consortium and the Wellcome Trust Case Control Consortium 2, 2010), identified eight new non-MHC loci (including *Endoplasmatic reticulum aminopeptidase 1* or *ERAP1* gene) which are implicated in the pathogenesis of psoriasis and these are linked to innate and adaptive immunity related-inflammatory pathways, as well as the skin barrier function. Furthermore, recently identified mutations on *Caspase-recruitment domain-containing protein 14 (CARD 14)* allele, located on *PSORS 2,* have been associated with an autosomal dominant familial form of psoriasis (Jordan et al., 2012). This gene is implicated in the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway which induces the production and release of key pro-inflammatory cytokines (IL-8 and chemokine ligand 20 or CCL20) that are responsible for initiating the cellular immune response seen in psoriasis (Jordan et al., 2012). Finally, results from a recent metanalysis of 3 GWAS and 2 independent immunochip (iChip) genomic databases identified 15 new psoriasis associated loci, as well as 5 new independent signals (Tsoi et al., 2012). These new loci were linked to T-cell regulation (such as *Runt-related transcription factor 3* or *RUNX3, T-cell activation GTPase activating protein or TAGAP and Signal transducer and activator of transcription 3 or STAT3*) and innate immunity, including interferon (IFN)-mediated antiviral defense (*DEAD box polypeptide 58 or DDX58*) and macrophage activation *(zinc finger CCCH-type containing 12C or ZC3H12C)*. These findings bring increased understanding of the pathogenesis of psoriasis.

1.5.2 The role of cellular and humoral immune response in psoriasis

The immune system encompasses the innate and acquired (adaptive) systems. Innate immunity is a quick, non-antigen specific immune response which serves as a first-line defense against pathogens. Innate immunity is controlled by a combination of physical and/or chemical barriers, keratinocytes and immune cells [natural killer (NK) cells, epidermal LC, DDC, neutrophils and macrophages]; (Sugita et al., 2007; Kobayashi and DeLeo, 2009; Hamerman et al., 2005); **Figure 1.11**.

Adaptive immunity is a specific, antigen-dependent, second-line defense system. Immunological memory is also generated. There are two subtypes of adaptive immunity; the cellular immune response controlled by T-lymphocytes and the humoral immune response mediated by B-lymphocytes (Bell, 2002); **Figure 1.11** and **Table 1.1** summarise the immune sentinels of the skin and their protective role in preserving the host integrity.

Considerable evidence exists that psoriasis is associated with dysregulation of both innate and adaptive immunity (Gaspari, 2006). Delayed type hypersensitivity reaction after epicutaneous allergen sensitisation (atopy patch testing) is decreased, whilst intradermal injection of purified protein derivative (PPD) shows a slow response in psoriasis patients (Moss et al., 1981). Interplay of inflammatory humoral factors (cytokines and chemokines) and various immune cells (T lymphocytes, DDC and keratinocytes) is present at the different stages of development of PP; **Table 1.2**.

Since the discovery of monoclonal antibodies, the epidermal and dermal inflammatory infiltrate of psoriasis has been thoroughly examined. T lymphocytes from PP are considered the key inflammatory cells which produce a pro-inflammatory, Th1/Th17 microenvironment that triggers and sustains the inflammatory process seen in psoriasis (Austin, Ozawa et al. 1999; Nestle, Di Meglio et al. 2009). The resulting milieu of pro-inflammatory cytokines (IFN-γ, TNF-α, IL-17A and IL-22) stimulates keratinocytes to continue hyperproliferation and secretion of additional pro-inflammatory mediators and thus maintain chronic inflammation (Nestle, Di Meglio et al. 2009). Interferon-γ and TNF-α are key mediators of inflammation in psoriasis and are secreted by both conventional (Th1 and Th17) and unconventional T cells [(Tγδ and natural killer-T cells (NKT)]; (Nestle, Di Meglio et al. 2009). These are implicated in the induction of inflammation as well as the exacerbation of psoriasis (Fierlbeck 1990; Kristensen, Chu et al. 1993; Pasparakis, Courtois et al. 2002).

The presence of anti-inflammatory, Th2 mediators (IL-4, IL-5, IL-10), in psoriasis, is scarce, indicating that the Th2-pathway may not contribute in the inflammatory process.

Activated epidermal T lymphocytes are hypothesized to represent the effector cells and this is driven by evidence from: a) the clonal expansion of only epidermal T cells in PP (Chang, Smith et al. 1994); b) the de novo development of PP, preceding the epidermal influx of T cells (Baker, Swain et al. 1984) and; c) the identification of a Th1/T cytotoxic (Tc) phenotype for these epidermal T cells (Austin, Ozawa et al. 1999). It is well established that in chronic PP lesions, these epidermal T-cells are mainly $CD3^+CD8^+$ T cells which produce Th1 cytokines to further stimulate keratinocytes, whilst promote chronic inflammation (Szabo, Hammerberg et al. 1998; Hijnen, Knol et al. 2012). These $CD3^+CD8^+$ T cells travel to the epidermis by expressing the very late antigen 1

(VLA1; also known as $\alpha_1\beta_1$ integrin); (Conrad, Boyman et al. 2007; Nestle, Di Meglio et al. 2009). The $\alpha_1\beta_1$ integrin is believed to be an essential factor for the formation of PP and is considered a possible therapeutic target for psoriasis. When $VLA1^+CD3+CD8^+$ T cells reach the epidermis, they express $\alpha_E(CD103)\beta_7$ which interacts with E-cadherin of keratinocytes and results in the retention of these effector T cells in the PP epidermis (Pauls, Schon et al. 2001). Consequently, the localised effector T cells produce IFN-γ and TNF-α which sustain the inflammation (Conrad, Boyman et al. 2007). In addition, it has been recently shown that $CD3^+CD8^+$ T cells from the peripheral blood of *HLA-Cw*06:02*$^+$ patients, by secreting IFN-γ, demonstrate increased response, to type 1 keratins and streptococcal M peptides, which have been shown to be potential "primary self or microbial antigens" implicated in the induction of the psoriasis (Johnston, Gudjonsson et al. 2004; Nestle, Di Meglio et al. 2009). This implies that *HLA-Cw*06:02* may potentially drive the epidermal influx of $CD3^+CD8^+$ T cells and thus psoriasis inflammation.

The role of epidermal $CD4^+$ T cells in psoriasis has also been explored. Intradermal injection of $CD4^+$ T cells in severe combined immunodeficiency (SCID) mice can induce plaque type-psoriasis lesions (Nickoloff and Wrone-Smith 1999). In addition, compared to chronic lesions, $CD4^+$ cells have been found to be more prominent in the epidermis of PP, in the early phase of development (Christophers et al., 2014). This epidermal influx induces the stimulation of antigen-specific $CD8^+$ cells (cross-priming) and their subsequent migration into the epidermis (Baker, Swain et al. 1984; Onuma 1994). Moreover, in chronic PP, there is evidence of a small proportion of $VLA1^+CD3^+CD4^+$ T cells reaching the PP epidermis. Although these cells are capable of producing Th1 cytokines, a high percentage is not secreting detectable amounts of pro-inflammatory mediators (Austin, Ozawa et al. 1999). This observation may be attributed to a non-functional role of these cells in the pathophysiology of psoriasis which leads to the inability of cytokine production and release as soon as they reach the epidermis or they may represent functionally deficient $CD4^+CD25^{high}$ T regulatory (Treg) cells (Sugiyama, Gyulai et al. 2005).

Table 1.1 Summary table of the immune cells of the skin, their surface markers and role in immune response.

Type of Cells	Antigenic markers on cell surfaces and functionality	Role
Langerhans' Cells (LC)	**CD1α** - A 49kD molecule associated with major histocompatibility complex (MHC) I and involved in LC differentiation **CD45** - A protein tyrosine phosphatise - Associated with MHC II - Involved in signal transduction - Also expressed on T-, B-cells and other antigen-presenting cells (APC) **CD4** - Interacts with MHC II molecules - Involved in signal transduction and activation of T-cells - Also present in the surface of T-helper (Th) cells and monocytes **Langerin** - Helps in the phagocytosis of the antigen by LC **S100** - Involved in inflammation signalling - Expressed by cells of neural crest origin	-Involved in antigen presentation and T-cells priming -Immunostimulatory properties -Reside in the epidermis and migrate to the lymph nodes
Dermal dendritic cells (DDC)	**CD1α and Langerin**	- Reside in the dermis - APC
T Lymphocytes • **T helper cells** - **Th1** - **Th2** - **Th17** • **Tregulatory cells** • **T cytotoxic (Tc) cells**	**CD3 and T-cell receptor (TCR)** - Specific markers for mature T lymphocytes **CD4** - The main cell surface antigen of Th1, Th2, Th17 and T regulatory (Treg) cells - T reg also express CD25 and FoxP3* protein **CD8** - Binds to gene products encoded in MHC I - Involved in cell signaling by enhancing antigen sensitivity	-Key cells in adaptive immune response -Recognise the antigen:MHC complex -Based on the cytokines they secrete, Th cells differentiate into Th1, Th2 and Th17 -Th1 secrete interferon (IFN)-γ and are involved in cellular immune response against intracellular pathogens and malignant cells -Th2 secrete interleukin (IL)-4, -5, -10 and -13 and induce the humoral (antibody-mediated) immune response against extracellular pathogens -Th17 secrete IL-17, -21 and -22 and are involved in inflammation and host-defence against extracellular pathogens. - Treg cells suppress immune responses and prevent autoimmunity -Tc cells show cytotoxic activity against intracellular pathogens and cancer cells

Natural killer (NK) cells	**CD56 and CD16** - NK phenotype is determined based on expression of CD56 and CD16; low (dim), high (bright) - Cytotoxic $CD56^{dim}CD16^{bright}$ NK cells; 90% - Immunomodulatory $CD56^{bright}CD16^{dim}$ NK cells; 10% **CD57** Controls maturation of $CD56^{dim}CD16^{bright}$ NK cells; - Also expressed on $CD8^{+}$ T-cells **CD161 and TCR Vα24, Vβ11** - Expressed on a NKT* cells; a subset of T-cells, which express NK surface antigen **CD158a / KIR2DL1*** - Inhibitory component of a paired KIR molecule; CD158h (KIR2DS1) is the activator component - Expressed on $CD56^{dim}CD16^{bright}$ NK cells	-Key players of innate immune response -Show direct, non-MHC, non-antigen-dependant cytotoxic properties against viruses and tumour cells.
Polymorphonuclear leukocytes (PMN)	**CD15** - Mediates signaling and chemotaxis of neutrophils, eosinophils and monocytes - Induces phagocytosis by neutrophils	-Effector cells of innate immunity -Show phagocytic properties against invading pathogens and chemoattract other immune cells
Mast Cells	**CD117 / c-kit proto-oncogen** - Regulates mast cell differentiation through activation of the tyrosine kinase pathway	-Key role in allergy and anaphylaxis -Involved in inflammation
Eosinophils	**CD15**	-Linked to allergy -Involved in immune response against parasites -Show antigen-presenting properties

Table 1.1 continued: Summary table of the immune cells of the skin, their surface markers and role in immune response.

** FoxP3= forkhead boxP3, NKT= natural killer T cells, KIR2DL= killer-cell imunoglobulin-like receptor 2 DL-1.*

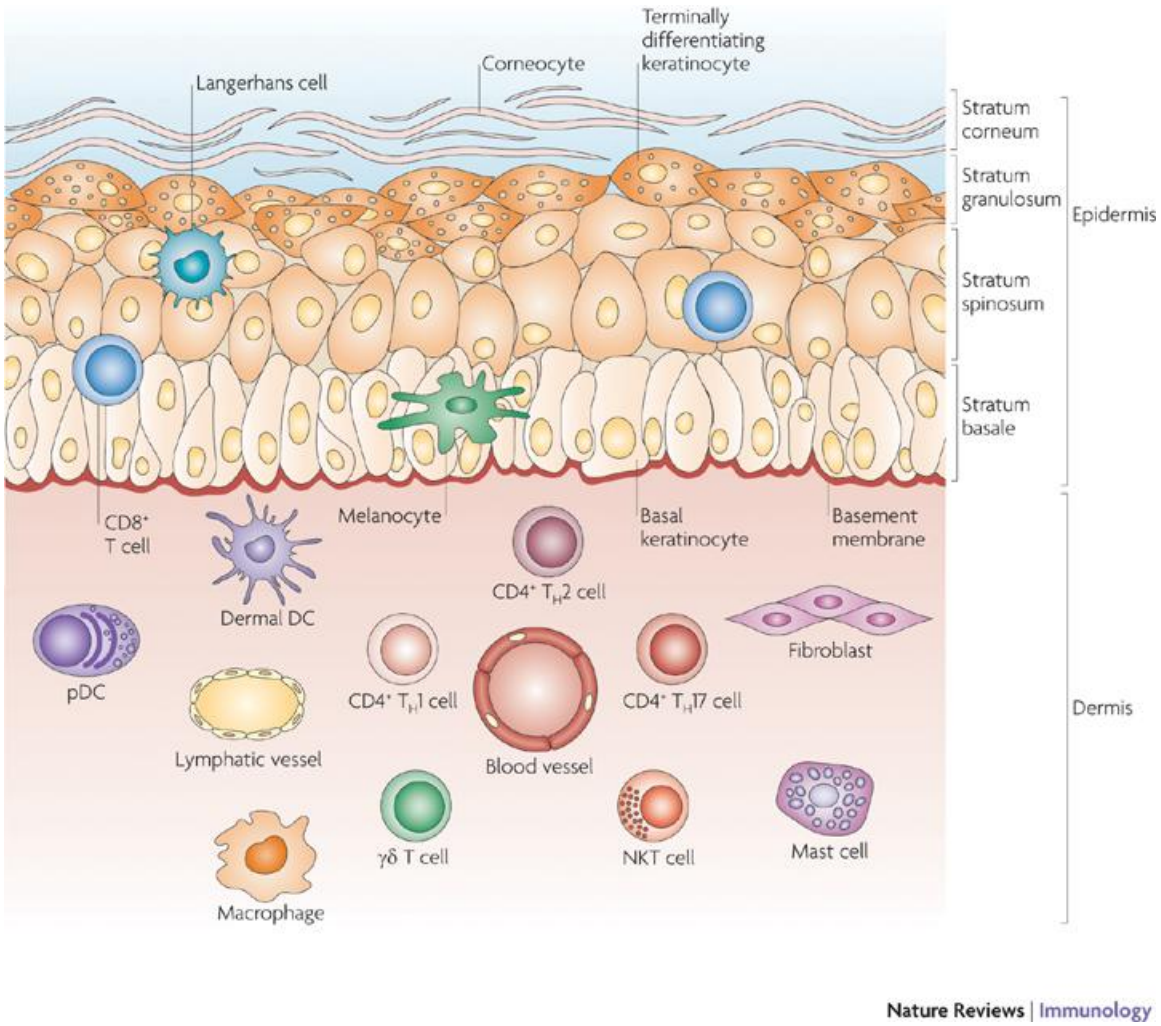

Figure 1.11 Immune cells of the skin.

Schematic showing the different types of cells involved in immunological responses, in the skin Figure obtained and reproduced from (Nestle et al., 2009)

**pDC=plasmacytoid dendritic cells, Th=T helper cells, NKT=natural killer T cells, DC= dendritic cells*

Cell types	PP	PN
Keratinocytes	• Abnormal hyperproliferation • Altered differentiation • Abnormal cellular signaling • Increased pro-inflammatory cytokine synthesis	• Normal or mildly increased proliferation with small areas of spongiosis in the epidermis (Koebner positive patients) • Normal differentiation • Abnormal cellular signaling • Increased expression of $\alpha_1\beta_1$ integrin
T lympocytes	• Increased T lymphocyte infiltration of the epidermis and dermis • Epidermal influx of $CD8^+$ • Increased production and secretion of pro-inflammatory cytokines and chemokines	• Mild to moderate dermal infiltration of T lymphocytes (Koebner positive patients)
Neutrophils	• Munro microabscesses in the epidermis	• Increased expression of $HLA\text{-}DR^+$ molecules
Langerhans' Cells	• Impaired migration to the local lymph nodes	• Impaired migration to the local lymph nodes • Increased expression of $HLA\text{-}DR^+$ molecules
Fibroblasts	• Increased proliferation	• Increased proliferation
Endothelial cells	• Increased expression of adhesion molecules	• Up-regulation of $HLA\text{-}DR^+$ molecules

Table 1.2 The main microscopic differences between involved (PP) and uninvolved (PN) skin.

This table shows the main histological and immunohistochemical differences seen in involved and uninvolved skin from psoriasis patients. Data were obtained from (Boer et al., 1994).

**HLA= human leukocyte antigen*

1.5.3 Environmental and psychological influences in psoriasis

Health is an interplay among biological-hereditary, environmental and psychological influences. In a similar way, psoriasis is primarily a multifactorial disease process, resulting from a combination of endogenous (genetic) and exogenous factors, including infections (streptococcal pharyngitis, human immunodeficiency virus-HIV infection), physical and psychological stressors (physical trauma, major life events, crises) and microstressors (daily hassle), weather changes (humidity, cold), lifestyle (smoking, alcohol, diet-obesity), hormonal changes (pregnancy, pos-partum period, menopause) and medications (beta-blockers, lithium, anti-malarial); (Ceovic et al., 2013; Chandran and Raychaudhuri, 2010; Elder et al., 2010; England et al., 1997; Patel and Weinberg, 2008; Raychaudhuri and Gross, 2000; Zhu et al., 2012).

The exact molecular mechanisms by which the aforementioned exogenous factors trigger or exacerbate psoriasis are not fully understood. *Human leukocyte antigen-Cw*06:02* positive patients with psoriasis are more prone in developing guttate psoriasis after streptococcal infection, while koebnarisation is common. In addition, *HLA-Cw*06:02* seems to play a major role in the development of psoriasis in HIV^+ patients (Mallon et al., 1998).

Although the distribution of *HLA-Cw*06:02,* as well as other genetic susceptibility factors (*LCE3B_LCE3C del*), covers a large number of ethnicities across the world, it is well known that there is a high incidence of psoriasis particularly in cold and humid environments, while the opposite is seen in dry places, where patients report higher improvement rates (Raychaudhuri and Farber, 2001; Riveira-Munoz et al., 2011).

Several studies discuss the role of excess smoking and alcohol consumption in the development or worsening of psoriasis. Some authors suggest that smoking is actually a triggering factor of psoriasis, while others claim that a higher numbers of cigarettes smoked per day can have a protective role for the disease (Kavli et al., 1985; Naldi et al., 1992). The theory behind smoking is that it can alter the function of PMNs, while, at the same time, induces the release of chemotactic mediators (Sonnex et al., 1988). Alcohol is another lifestyle factor that has been implicated in the pathogenesis of psoriasis. It is well documented that psoriasis patients that consume large amounts of alcohol, report a higher incidence of disease flares (Farkas and Kemény, 2010; Kirby et al., 2008; Zhu et al., 2012). It is however unclear the exact mechanism of action. One possible explanation would be that alcohol promotes central obesity, which leads to glucose intolerance and a higher risk for T2DM (Dubreuil et al., 2014). Glucose intolerance is linked to an increased production of pro-inflammatory cytokines which are also key factors in the pathogenesis of psoriasis (Gisondi et al., 2007; Gottlieb et al., 2008; Shapiro et al., 2007). In addition, alcohol can influence the immune system in many ways, including the function of both inflammatory cells and keratinocytes (Farkas and Kemény, 2010).

Another explanation is that psychological stress is directly linked to certain lifestyle habits, such as smoking, alcoholism and obesity. Emotional stress is a well-documented trigger for inflammatory skin diseases (Buske-Kirschbaum and Hellhammer, 2003; DeWeerdt, 2012; Heller et al., 2011). Stress induces the activation of the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenomedulary (SMA) axes, both of which regulate the cutaneous immune response. Normal "fight or flight" response to stress stimulates the release of stress hormones (cortisol, adrenaline and norepinephrine), which have a protective role. When there is a stress system malfunction, then the homeostasis between HPA and SMA axes is lost, which leads to upregulation of pro-inflammatory mediators in the skin (Huynh et al., 2013).

Taken together, it is likely that psoriasis patients inherit only a predisposition to the disease, that still requires an exogenous stimulus to express its phenotype (Ortonne, 1999).

1.6 Psoriasis associated comorbidities

Epidemiological data have indicated an increased risk of various chronic and serious health conditions, also known as "comorbidities", for patients with psoriasis (Augustin et al., 2010). Recent studies support that compared to the general population and patients with mild psoriasis, the life expectancy of patients with early onset and severe psoriasis is significantly reduced, due to a higher risk for multiple comorbidities (Gelfand et al., 2007; Gulliver et al., 2011). These comorbidities can be divided into: a) those with similar genetic component and

immunopathogenesis to psoriasis and; b) others, mainly metabolic, which are related to the chronicity of the disease (Christophers, 2007).

Comorbidities with shared genetics include PsA, Crohn's disease, T2DM, metabolic syndrome and ischaemic heart disease (IHD); (Mallbris et al., 2006; Quaranta et al., 2009; Lu et al., 2013; Martinez-Borra et al., 2003; Cargill et al., 2007). Disease severity and early age of onset have also been linked to an increased risk of myocardial infarction, stroke, atrial fibrillation and premature cardiovascular death (Naldi et al., 1992; Mallbris et al., 2004). What is not clear is whether psoriasis itself is an independent risk factor for these cardiovascular events and whether early intervention and / or more aggressive treatment of psoriasis will alter the frequency of these events.

In addition, patients with psoriasis have been reported to be at an increased risk for skin cancer, lymphomas, liver and kidney disease (Alderson and Clarke, 1983; Rosenberg et al., 2007). However, there is no clear evidence whether psoriasis alone or the long-standing treatments are inducing this particular susceptibility to malignancy.

Psychological disability, secondary to stigmatisation, is also common in psoriasis (Gupta, 2010; Naldi et al., 1992). Compared to the general population, an increased risk for depression, anxiety disorders, substance abuse and suicidality has been recently reported for young, male patients with severe psoriasis (Olivier et al., 2010). Accumulative evidence exists that the chronic course of psoriasis negatively impacts patients' lives including social activities and daily living, as well as their response to treatment (Fortune et al., 2002; Fortune et al., 2003). Other studies suggest that control of psoriasis with pharmacological as well as non-pharmacological interventions is linked to an improvement in symptoms of psychological morbidity (Tyring et al., 2006).

1.7 Management of psoriasis

The management of psoriasis is based on the clinical severity of the disease. A step-wise approach is employed, with topical therapy (corticosteroids, calcipotriol, tazarotene, tar, anthralin and keratolytics) as first line for localised, mild to moderate disease. Phototherapy [broadband (290 – 320 nm) ultraviolet light B (UVB), narrowband (311 nm) UVB and psoralen plus ultraviolet light A (PUVA)] for moderate to severe disease and then systemic therapy [retinoids (acitretin), methotrexate, cyclosporine and fumaric esters] for severe extensive disease (second line treatment); (van de Kerkhof, 2001).

Over the past decade, new insights into the immunopathogenesis of psoriasis have resulted in the development of various biologic agents, which target key molecules specific to the inflammatory process of psoriasis. These are the T-cell modulating agents (alefacept, efalizumab), inhibitors of TNF-α (adalimumab, etanercept, and infliximab) and the inhibitor of IL-12 and IL-23 (ustekinumab); (Weger, 2010). These agents are administered to severe psoriasis patients with significant psychosocial impairment due to their skin disease (third line treatment).

Despite the wide range of therapeutic agents, psoriasis remains a difficult to control disease. Novel targets for future therapeutic agents are under investigation in clinical trials and

include IL-17 antagonists, IL-20/22 antagonists, janus kinase (JAK) and phosphodiesterase 4 (PDE4) inhibitors (Patel et al., 2012).

1.8 Other phenotypic classifications of psoriasis

The identification of psoriasis-risk genes and the discovery of the Th17 pathway have shed light on the pathogenesis of psoriasis. Consequently, many authors have tried to reclassify the disease according to a combination of clinical and non-clinical predictors such as genetics, age of disease onset and disease duration. **Table 1.3** summarises all recent studies on the various proposed classifications of psoriasis. Briefly, these are the following:

- The classification proposed by Christensen and coworkers, which classifies psoriasis based on depth of induration of PP lesions (Christensen et al., 2006).

- The recognition of two phenotypic patterns of psoriasis in active (unstable) and inactive (stable), based on the disease activity and severity (Griffiths et al., 2007b; Griffiths and Barker, 2007a; Naldi and Gambini, 2007).

- The identification of two subtypes of psoriasis based on the average size of psoriasis plaques (Lew et al., 2004); this classification was based on the dynamic behaviour of psoriasis plaques (small or large), the different susceptibility haplotypes of psoriasis and patients' ethnicity.

- The classification proposed by the French psoriasis research group; psoriasis is divided in six subgroups, based on clinical and demographic parameters. These parameters are the area of skin involvement, the presence of guttate or pustular lesions and the susceptibility to environmental stimuli (Guinot et al., 2009).

- Of particular relevance to this thesis is the classification of psoriasis in two subgroups, by Henseler and Christophers, according to the age of onset of the disease; type I or early onset psoriasis (EOP), occurring before the age of 40 years, and type II or late onset psoriasis (LOP), affecting patients over 40 years of age (Henseler and Christophers, 1985).

Table 1.3 Summary of studies on the different classifications of psoriasis.

Year of publication / Author	Parameters of classification	Phenotypic subgroups
1808: R. Willan (reviewed in Bechet, 1936) **1819:** T. Baterman (reviewed in Bechet, 1936)	Pattern of primary lesion	• psoriasis Guttata (Guttate) • psoriasis Diffusa (Plaque) • psoriasis Palmaria (Palmar) • psoriasis Inveterate (Erythrodermic?)
1888: R. Crocker (reviewed in Barber and Eyre, 1927) **1890:** F.H. Hallopeau (reviewed in Barber and Eyre, 1927) **1910:** Von Zumbusch (reviewed in Barber and Eyre, 1927) **1930:** H.W. Barber (Barber and Eyre, 1927)	Presence of pustules	• Dermatitis Repans • Acrodermatitis Suppurativa Continua • Generalized Pustular psoriasis • Acrodermatitis Perstans and Pustular psoriasis
1985: T. Henseler and E. Christophers (Henseler and Christophers, 1985)	Age of onset of psoriasis	• Type I or early onset psoriasis-EOP (≤ 40 years old) • Type II or late onset psoriasis-LOP (>40 years old)
2000: J.G. Krueger and co-workers (Krueger et al., 2000)	Disease severity	• Mild psoriasis • Moderate psoriasis • Severe psoriasis
2004: W. Lew, E. Lee and J.G. Krueger (Lew et al., 2004)	Size of psoriatic plaque	• Small plaque psoriasis (≤3 cm in size) • Large plaque psoriasis (>3 cm in size)
2006: T.E. Christensen and colleagues (Christensen et al., 2006)	Induration of psoriatic plaque	• Thin plaque psoriasis (<0.05 mm of plaque elevation) • Intermediate plaque psoriasis (0.05 – 0.1 mm of plaque elevation) • Thick Plaque psoriasis (>0.1mm of plaque elevation)
2007: International Psoriasis Council (Griffiths et al., 2007b)	1) Clinical pattern of psoriatic plaques 2) Location 3) Disease Activity	• Localised forms of psoriasis or • Widespread forms of psoriasis +/- • Stable / Unstable psoriasis • EOP / LOP • Small / large plaque • Thick / Thin • Nail psoriasis • Follicular psoriasis
2008: J.E. Osborne and P.E. Hutchinson (Osborne and Hutchinson, 2008)	Basal area of skin involvement (A_{basal})	• Phenotype A with low A_{basal} and high flare score • Phenotype B with high A_{basal} and low flare score
2009: French psoriasis Research Group (Guinot et al., 2009)	1) Extend of skin involvement 2) Presence of guttate or pustular lesions 3) Sensitivity to environmental factors 4) Familiar history 5) Age of onset of psoriasis	• Type 1 • Type 2 • Type 3 • Type 4 • Type 5 • Type 6

1.9 Early and late onset psoriasis

The stratification of psoriasis by age of onset is investigated in this thesis, on the basis of clinical, histological and immunohistological criteria.

1.9.1 Defining the age of onset

Previous studies have encountered the age of psoriasis onset as an important epidemiological criterion in the investigation and interpretation of psoriasis (Economidou et al., 1985; Henseler and Christophers, 1985; Ingram, 1954). Consequently, a clear definition of the term "age of disease onset" is required in this thesis; many authors have used the age at which their participants recall the first symptoms of their psoriasis, while others use the age at which their participants were first diagnosed (by a consultant dermatologist or general practitioner-GP) with the disease. Langley and colleagues identified the inconsistency of data seen in most epidemiological studies, between the reported (by the participant) age of psoriasis onset and the actual date of diagnosis of the disease from a health practitioner (Langley et al., 2005). The previous could be explained on the basis that many psoriasis patients, with early lesions on the scalp, remain undiagnosed until the rash spreads to involve other parts of the body. For the purpose of this study, the age of disease onset is defined as the age at which the patient was diagnosed with psoriasis by a consultant dermatologist or a general practitioner. The date on which the patient recalls their first symptoms was also taken into consideration, and if that date occurred over one year prior to the date of diagnosis, the subject was excluded from the study.

1.9.2 Clinical differences between early and late onset psoriasis

Psoriasis may occur at any age, from birth to senility (Buntin et al., 1983). A bimodal distribution in the age of onset of psoriasis has been extensively discussed in the literature, mainly for plaque psoriasis, with more limited data on pustular psoriasis (Lyons, 1987). The first reports of two peaks of age of plaque psoriasis onset were published by Ingram, in a sample population of 1,356 patients from the United Kingdom (UK); (Ingram, 1954). The author recognized a peak at pubertal years and a second peak at the 4^{th}-5^{th} decade of life and associated this bimodal distribution of the age of onset with the menarche and menopause of his female patients.

Henseler and Christophers' epidemiological study of 2,147 psoriasis patients from Germany, classified plaque psoriasis based on the age of onset of the disease (Henseler and Christophers, 1985) into two distinct disease types; EOP or type I psoriasis and LOP or type II psoriasis. Presentation of EOP was characterised by a younger onset age (≤ 40 years), a higher incidence of a guttate phenotype, more frequent exacerbations and often relapses, usually following β-hemolytic streptococci infections, a higher incidence of koebnerisation, an increased psychological impact, while nail and facial involvement were commonly seen. In addition, EOP patients were more likely to have a first degree relative with psoriasis and a more aggressive clinical course of the disease. Late onset psoriasis, with first symptoms usually after the age of 50

years, presented with a milder and more stable disease course and a lower psychosocial impact. Moreover, a family history of psoriasis was less frequent in LOP patients.

In 1995, Swanbeck and colleagues' study described a late onset phenotype, occurring after the age of 50 and mainly affecting female patients. In line with Ingram's observations, the onset of plaque psoriasis of the female participants coincided with their menopausal transition phase (Swanbeck et al., 1995). Again, these findings further underscore the importance of hormonal influences in the onset of plaque psoriasis, especially in LOP patients.

A few studies, investigating the age of onset, have been conducted on pustular psoriasis. Baker and Ryan, using 104 cases of GPP, identified two subtypes according to the age of onset of the pre-pustular stage (the appearance of the first psoriasis plaque); (Baker and Ryan, 1968). Type I pustular psoriasis, therefore, referred to an early onset of the first clinical signs of psoriasis and a prolonged pre-pustular stage. Type II pustular psoriasis consisted of patients with late onset disease, atypical clinical presentation of the pre-pustular skin lesions and a rapid disease evolution.

A large-scale observational study conducted in Spain, confirmed the findings of Henseler and Christophers for plaque psoriasis; the authors showed that, as the age of onset of psoriasis increased, there was a progressive decrease in the incidence rates of a positive family history of psoriasis (Ferrandiz et al., 2002). Their results along with Henseler and Christophers pointed towards two different psoriasis subtypes: a subtype with a strong genetic background for the disease (EOP) and one in which genetic factors play a secondary role (LOP); (Stuart et al., 2002).

Another study from Sweden of 400 psoriasis patients, stratified clinical data by age of onset and presence or absence of guttate psoriasis (Mallbris et al., 2005). As pointed out by the authors, patients with guttate psoriasis, were more likely to experience the guttate onset early in life (≤40 years of age), usually after an episode of streptococcal pharyngitis, whilst they were more prone to atopic eczema in childhood. On the other hand, the non-guttate group of patients mostly included patients with late onset disease (>40 years of age), with a higher prevalence of nail psoriasis and PsA.

Many other studies, from around the world, have explored the bimodal distribution of the age of onset of plaque psoriasis. While studies on Caucasian populations show a younger age at onset, especially between the 19^{th}-25^{th} year of age, epidemiological studies on Asian populations show a predisposition to LOP (Economidou et al., 1985; Schmitt-Egenolf et al., 1996). This is particularly interesting in the current context, as it shows that LOP is less frequent in the Western world. More specifically, a study of 607 psoriasis patients from northern Japan showed that the mean age of onset of psoriasis of their study population was 40 years (Takahashi et al., 2009). A large epidemiological survey on 28,628 psoriasis patients from Japan, also showed a higher mean age of onset (39 years) compared to that seen in Caucasian populations (Kawada et al., 2003). Similar findings were recently reported by a Korean group of researchers who divided plaque psoriasis in three groups; the early-, the middle-age and the elderly-onset group (Kwon et al., 2012). The authors actually concluded that elderly onset psoriasis (>60 years) may be considered as a distinct entity, as its clinical presentation differs from the other two subtypes. More specifically, scalp psoriasis was more prevalent in the elderly onset age group while extensor surfaces of the skin were less affected. Other studies on Indian, Malaysian and Taiwanese populations have similarly demonstrated that LOP is more prevalent in these countries (Yap, 2010; Betsy et al.,

2011; Yun-Ting et al., 2009). These particular observations point towards LOP being more common in certain ethnic groups. Consequently, as people of the same ethnic group often share the similar genes, passed down from common ancestors, the above findings may hint at a different genetic basis of LOP compared to EOP.

1.9.3 Genetic differences between early and late onset psoriasis

Henseler and Christophers were the first to show genetic differences between EOP and LOP, noting that *HLA-Cw*06:02* was present in approximately 85% of EOP patients compared to 15% of LOP patients, a frequency which is actually comparable to that found in the general population (Henseler and Christophers, 1985; Henseler and Christophers, 1998). Subsequently, Szczerkowska-Dobosz confirmed the lack of any link between *HLA-Cw*06:02* and LOP (Szczerkowska-Dobosz et al., 2007). A Chinese research group also confirmed that *HLA-Cw*06:02* and *ERAP1* was more commonly seen in Chinese EOP patients compared to LOP patients (Sun et al., 2010). Another recent study from Sweden, explored the presence or absence of *ERAP1* and *HLA-Cw*06:02* on EOP and LOP patients; it was found that, in addition to *HLA-Cw*06:02* , *ERAP1* is also not as frequent in LOP as in EOP (Lysell et al., 2013). Their findings confirmed these presented by Sun et al, on Chinese psoriasis patients (Sun et al., 2010). Gudjonsson and colleagues carried out a cohort study of 1,019 Caucasian patients, aiming to define the clinical and demographic features of *HLA-Cw*06:02* positive and *HLA-Cw*06:02* negative psoriasis patients (Gudjonsson et al., 2006a). They found that the *HLA-Cw*06:02* positive patients experienced an earlier disease onset and suffered a more severe disease as well as more frequent flares of their psoriasis, usually post-URTIs. The *HLA-Cw*06:02* negative patients were more likely to develop a milder form of psoriasis, usually occurring later in life and affecting the scalp and nails. Additionally, PsA was more common in these patients.

Recent research has shown that there are additional genetic haplotypes which differ between EOP and LOP (Queiro et al., 2013). A study carried out in 156 psoriasis patients from Denmark demonstrated an increased frequency of *HLA-B13* and *-B17* in EOP patients, regardless of gender (Svejgaard et al., 1974). A UK-based study of 597 EOP patients, looking at the association of previously reported, psoriasis risk SNPs in the *IL-12B* and *IL-23R* genes with psoriasis, showed that these genes are definitely playing a key role in the pathogenesis of EOP (Smith et al., 2008a). One of the shortcomings of the study is that it did not include LOP patients. Interestingly, a study from Germany of 156 EOP, 75 LOP and 345 healthy volunteers, observed a significant association of familial EOP (especially in male EOP patients) with carriage of the *TNFA* promoter polymorphism, *TNFA238*2* (Reich et al., 2002). A very recent metanalysis has confirmed the previous findings, whilst the authors demonstrated that these *TNFA* polymorphisms are not associated with LOP (Jia et al., 2013). When Reich and co-workers investigated polymorphisms in the gene encoding for IL-1β *(IL1B gene*) and its antagonist, IL-1 receptor antagonist (IL-1Ra and *IL1RN gene*), they detected a significant association of LOP with the *IL1B-511*1/1* homozygous genotype (Reich et al., 2002). Interestingly, on a functional level, using cell cultures, carriers of the *IL1B-511*1/1* genotype, demonstrated an increased production of IL-1Ra, in response to IL-10 and lipopolysaccharide (LPS). Their findings were very recently replicated by Hebert and co-workers

(Hebert et al., 2013). Taken together, the aforementioned observations indicate that allelic variants in cytokine genes are associated with the age of onset of psoriasis, as well as point out potential differences at a pathomechanistic level between EOP and LOP.

Moreover, a GWAS of patients of European ancestry has identified a deletion in the *late cornified envelop (LCE)* gene cluster, comprising the absence of *LCE3B* and *LCE3C* (*LCE3C_LCE3B-del*), and which is linked to a high risk for psoriasis (De Cid et al., 2009). The *LCE3C_LCE3B-del* is associated with an impaired skin barrier formation and repair. A multicentre meta-analysis, including European and certain Asian populations (Chinese, Japanese and Mongolian), demonstrated that *LCE3C_LCE3B-del* is a common genetic variant that increases susceptibility to psoriasis (Riveira-Munoz et al., 2011). A Chinese research group, which investigated the same *LCE deletions* on patients from Northern China, also observed that the *LCE3C_LCE3B-del* was significantly more frequent in EOP patients, compared to LOP (Xu et al., 2011).

More studies on Asia populations revealed a SNP in the proximity of the *involucrin (IVL)* gene, within *PSORS4*, with an increased frequency in Singaporean Chinese, EOP patients (Chen et al., 2008). This was not, however, observed in psoriasis patients of European ancestry (Kainu et al., 2009).

Another study from Korea observed significant links between polymorphisms in *IL-2* and *IL-4 genes* and LOP patients, compared to controls (Kim et al., 2007). The previous results indicate a potential imbalance of Th1/Th2 cytokines between EOP and LOP in certain ethnic groups. Polymorphisms in the *serotonin 2A receptor (5-HTR2A)* have been linked with LOP, Thai patients (Ronpirin et al., 2010). An increased frequency of *the macrophage migration inhibitory factor (MIF)-173C allele* was observed in LOP, male patients of Chinese origin, but was different of the frequency found in Caucasian populations (Wu et al., 2009). Late onset psoriasis patients of Mediterranean origin, especially female, were found to have a significantly higher frequency of particular SNPs in *Notch4 gene*, while SNPs in *Notch2* were more prevalent in EOP, male subjects (Michailidis et al., 2013). Finally, the frequency of *HLA-Cw7* was higher in EOP patients from Turkey, compared to EOP, who were found to have an increased frequency of *HLA-Cw6, -A30, -B50,* and *-DR7 alleles* (Kundakçi et al., 2002). The aforementioned findings suggest the presence of various polymorphisms associated with LOP patients of different ethnic background. However, these results derive for relatively small numbers of patients, while the majority of the findings have not yet been replicated (Queiro et al., 2013).

1.9.4 Immunological differences

In an attempt to explore differences in the inflammatory microenvironment of EOP and LOP, Craven and coworkers studied polymorphisms in IFN-γ, IL-10, IL-4 and TNF-α, in 84 psoriasis patients (49 EOP and 35 LOP); (Craven et al., 2001). When comparing LOP subjects with a control population, the authors found that there was a slightly increased frequency of the heterozygous (*G/A*) genotype of the gene encoding the anti-inflammatory IL-10. On a functional level, the heterozygous *G/A* genotype indicates a moderate production of IL-10, while the homozygous *G/G* and *A/A* genotypes relate to high and low production of IL-10 respectively. In

general, low levels of IL-10 have been associated with the upregulation of anti-inflammatory (Th1-derived) cytokines (Wongpiyabovorn et al., 2008). Kingo and colleagues tried to replicate Craven's results in a total of 248 Estonian patients but failed to detect any statistically significant link between the age of psoriasis onset and the haplotype distribution of IL-10 SNPs (Kingo et al., 2003). However, they showed that the *ACC proximal haplotype of IL-10* gene was more frequent in those patients with a mild form of psoriasis. Wongpiyabovorn and colleagues proposed that the *AAGC distal haplotype of IL-10* gene (low levels of IL-10) is more likely to be found in Thai LOP patients (Wongpiyabovorn et al., 2008). Taken together, the previous findings further support the hypothesis of differences in the underlying pathogenesis of EOP and LOP.

Shaw and colleagues strengthened the hypothesis of EOP and LOP as having different immunopathogenesis (Shaw et al., 2010). The authors showed that, after injecting IL-1β and TNF-α in PN skin, the migration of epidermal LC is different in these two subtypes of psoriasis. Specifically, in the EOP group, LC did not migrate from the epidermis, after injecting either intralesional IL-1β or TNF-α. On the other hand, the LOP group showed migration of LC in response to intralesional IL-1β, while there was no response to intralesional TNF-α.

1.9.5 Identifying the knowledge gap

The previous sections imply that EOP and LOP are clinically, genetically and immunologically different. This section will identify the knowledge gap which this thesis will attempt to address.

Although recent observational studies have demonstrated that there is a strong relationship between psoriasis and other chronic and disabling conditions, it is not clear whether these comorbidities are linked to both or either EOP or LOP. One example is T2DM, which has recently been linked to psoriasis (Shapiro et al., 2007; Armstrong et al., 2012). Interestingly, a recent study from Spain, of 661 psoriasis patients and 661 aged-matched controls, explored the potential of a link between T2DM in EOP and LOP patients; the authors found that there was a higher risk to develop T2DM in the non-familial, *HLA-Cw*06:02* , LOP group (Armesto et al., 2012). A similar study of 2,267 psoriasis patients from Malaysia, found that T2DM is more prevalent in LOP patients of Asian ethnicity. Both studies suggest important differences in associated comorbidities between EOP and LOP, in different ethnic groups, but there is still need of additional, high quality studies, to confirm the previous results and identify potential, additional links between other psoriasis associated comorbidities and the aforementioned subtypes of psoriasis. A new study from Hungary, using a multivariate analysis model, has recently linked central obesity (increased waist circumference) with LOP only, while they were also able to detect a trend for a higher prevalence of T2DM in LOP, compared to EOP patients (Herédi et al., 2013).

Moreover, there is comparatively little data on the relative psychosocial impact of EOP or LOP on patients' lives. Early onset psoriasis has been previously associated with greater psychological distress, including high levels of anxiety and depression, which accelerates and worsens the clinical course, whilst leads to ineffective coping of the disease (Gupta et al., 1996). Recent data from 101 Swedish patients with psoriasis, suggest that EOP, especially patients <20 years old, are more likely to express certain personality traits, more susceptible to environmental

stress and hence further impair the psychological adjustment to psoriasis (Remröd et al., 2013a). On the other hand, Kotrulza and colleagues, from the University of Croatia, recently conducted an observational study comparing 70 psoriasis patients (44 with EOP and 26 with LOP) with 70 control patients, suffering from other non-psychosomatic skin problems (Kotrulja et al., 2010). Contrary to the results of previous studies, the authors found that the LOP had higher levels of depression and a more severe disease course, compared to the EOP group . On the basis of the previous studies, it is interesting to further explore relations between age at psoriasis onset and psychological disability.

In addition, there are only 3 studies on differences in the treatment between EOP and LOP. Ferrandiz and colleagues were the first to show that compared to EOP, LOP patients receive different therapeutic management (Ferrandiz et al., 2002). More specifically, EOP patients were more likely to have tried systemic treatment compared to LOP patients. This was attributed to EOP being a more severe skin disease from LOP. The second study was from Israel. The authors highlighted the significant improvement effect of climatotherapy in the Dead Sea in the EOP patients compared to LOP patients, irrespective of disease severity (Harari et al., 2012). More recently, a study from Italy demonstrated that EOP is an independent predictor of use of biologic agents, although it is not clear whether this association was produced due to the longer duration of the disease in their sample of biologic vs non-biologic users (Di Lernia and Ficarelli, 2012). There remains a great deal more to be explored on this topic.

Furthermore, it is well established that histology helps distinguish between the different clinical phenotypes of psoriasis. Krengel et al, for example, have studied the histological structure of PP from plaque, guttate and pustular psoriasis and identified various morphological similarities, as well as key differences which distinguish between phenotypes (Krengel et al., 1998). Currently, although EOP and LOP are recognised as different subphenotypes of psoriasis, there are no comparative studies of their histology.

Moreover, despite recent evidence of immunological differences between EOP and LOP, there are no other studies investigating potential variations in pathomechanism, which may be driven by the different genetic background of these two entities (Shaw et al., 2010).

The Henseler and Christophers' classification of psoriasis is an interesting addition to psoriasis research. There is now significant evidence that EOP and LOP are distinct, with a diverse pattern of gene expression, clinical picture and a different pathogenesis. Previous research has therefore paved the way for further investigation of these two subtypes of psoriasis, which will be discussed in the next chapters of this thesis.

1.10 Summary

Despite psoriasis being a common skin disorder, no specific diagnostic test exists either to confirm the clinical diagnosis or predict future appearance of the disease. The diagnosis of psoriasis is almost always made on the basis of clinical findings. Therefore, most of the classification schemes that exist for psoriasis are based on the morphology of PP lesions and clinical pattern. Recent advances in the genetics of psoriasis have permitted the identification of

risk genes associated with psoriasis and have shed light on the pathogenesis and aetiology of the disease. Consequently, various authors have tried to propose new models for reclassifying psoriasis and enhance diagnosis and treatment.

An identification of two subtypes of psoriasis based on the “age of disease onset” has been described by Henseler and Christophers (Henseler and Christophers, 1985); EOP (≤ 40 years of age) and LOP which includes patients whose psoriasis mainly developed after the age of 50. Multiple studies have shown that these two subtypes are clinically, genetically and immunologically heterogeneous. More research into this area is required to thoroughly explore differences between EOP and LOP and thus, identify the basis of these differences (**Table 1.4**).

Table 1.4 Summary of known and missing criteria which distinguish early onset-EOP and late onset psoriasis-LOP.

Criteria	Early onset psoriasis	Late onset psoriasis
Clinical pattern of skin lesions	• Plaque psoriasis, mainly affecting extensor surfaces • Other frequent phenotypes; guttate psoriasis and erythroderma • Not explored in detail	• Plaque psoriasis, mainly affecting scalp and nails • Not explored in detail
Social impact	• Not clear in the literature	• Not clear in the literature
Psychological impact	• Depression • Psychological traits of anxiety, embitterment, mistrust, irritability and verbal aggressiveness • Not clear in the literature	• Depression • Anxiety • Not clear in the literature
Psoriasis associated comorbidities	• Reported links with childhood atopic eczema • Cardiovascular disease (CAD) • Not fully explored	• Reported links with type 2 Diabetes Mellitus, central obesity, psoriatic arthritis and CAD • Not fully explored
Therapeutic management	• Significant improvement with Dead sea climatotherapy • More frequent use of systemic and biologic therapies • Additional information on other treatments is required	• Not fully explored
Genetic markers	• *Human leukocyte antigen (HLA)-Cw*602*; positive in 55-80% of patients • Tumour necrosis factor- alpha (TNF-α) promoter polymorphisms	• *HLA-Cw*06:02*; positive in 15% of patients • Significant link with the interleukin (*IL)1B-511*1/1* homozygous genotype
Histopathological elements	• Not known	• Not known
Inflammatory microenvironment	• No migration of epidermal Langerhans' cells (LC) upon stimulation with interleukin (IL)-1β or TNF-α • Not fully explored	• Migration of epidermal LC upon stimulation with IL-1β only • Not fully explored

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1.11 Hypothesis, aims and objectives

The hypothesis upon which this thesis was based is the following; EOP and LOP are different clinical, histological and immunological entities. More specifically:

1. LOP is clinically different to EOP and is not linked to the same comorbidities.
2. LOP has a different inheritance pattern compared to EOP.

3. LOP has a different psychosocial impact on patients compared to EOP.
4. LOP has a different immunopathogenesis compared to EOP and hence there exist differences in the components of the cellular infiltrate of the epidermis and dermis of PP and PN skin.

The aim of this thesis was to identify any clinical, histological and immunological differences between EOP and LOP. The specific objectives of the study were:

1. To confirm existing phenotypic differences between these two subtypes of psoriasis in the literature.
2. To investigate if the association of other medical conditions with psoriasis is specific to EOP alone.
3. To identify any particular clinical phenotype specific to either EOP or LOP.
4. To explore differences in the use and response to different treatments, used in the management of EOP and LOP.
5. To identify histological differences and similarities between EOP and LOP.
6. To investigate the immunohistochemical distribution of key inflammatory cells in PP and PN skin of EOP and LOP.

1.12 Study design

Three separate studies were conducted to further investigate and identify the potential aforementioned phenotypic differences between EOP and LOP; a questionnaire study to explore potential clinical differences, a histological study to identify potential histological differences and an immunohistochemistry (IHC) study to look into immunological differences between the aforementioned types of psoriasis.

The study design, methods and results will be described in detail in subsequent chapters of this thesis.

2. Materials and methods

This chapter comprises the detailed methodologies of the questionnaire, the histology and IHC study. Additional information on the protocols and tools of this thesis are included in the Appendix section (**Appendices A and B**). Summaries of methodologies have been included in each chapter.

2.1 Clinical and psychological evaluation of early and late onset psoriasis

2.1.1 Study design

An observational clinical study was carried out to examine for differences in the demographic, clinical and psychological features of EOP and LOP between November 2010 and December 2012. The study was composed of two cohorts; a prospective cohort of 112 psoriasis patients and a retrospective cohort of 239 psoriasis patients. A control group of 157 non-psoriasis subjects was recruited retrospectively **(Figure 2.1)**.

2.1.2 Prospective cohort

This arm of the study required a one-hour visit at the Dermatopharmacology Unit, Salford Royal National Health Service (NHS) Foundation Trust (SRFT), Salford, UK. Enrolled subjects were asked to complete a study specific questionnaire (**Appendix A**). All subjects had a physical examination and skin assessment performed by the study physician (Eleni Theodorakopoulou; E.T.).

2.1.2.1 Study population

Psoriasis volunteers were identified from either the weekly psoriasis clinic, the outpatient Dermatology department, Dermatology ward and Phototherapy Unit, at SRFT. The study posters were displayed in relevant sites, at SRFT. In addition, electronic versions of the study posters were displayed at the student portal of the University of Manchester and in the newsletter of the Dermatopharmacology Unit. All volunteers provided written, informed consent prior to any study procedures. The eligibility of participants was established via a telephone call and was confirmed at the study visit. In keeping with good clinical practice (GCP) international guidelines, provided by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), potential participants were given a verbal description of the study and a written information leaflet via post or email, prior to screening. Participants were given at least 24 hours to read the study literature and consider taking part in the study.

Enrolled patients of the prospective cohort, based on the age of onset of their psoriasis, were categorised into two groups; the EOP group (age of onset≤40 years of age) and the LOP group (age of onset ≥50 years of age).

2.1.2.2 Ethical approval and study specific training

Ethical approval was obtained from the North West 10 Research Ethics Committee (REC), on 26th October 2010 (*REC reference number: 10/H1011/68*) and the study was conducted in compliance with the declaration of Helsinki guidelines. Research and Development (R&D) and site specific approval were obtained by the R&D department of SRFT, on the 2nd of November 2010. Recruitment officially started on the 3rd of November 2010.

The study doctor (ET), who was GCP trained, was experienced in assessing psoriasis severity with the psoriasis area and severity Index (PASI); (Fredriksson and Pettersson, 1978). Additional training on PASI was provided by Professor Christopher Griffiths, during the weekly psoriasis clinic at SRFT, whilst an online PASI training course was completed (the GRAPPA video project, by Kristina Callis Duffin). Specific training on th evaluation of PP elevation, using the National Psoriasis Foundation (NPF) induration card score, was also provided by Professor Christopher Griffiths. However, the NPF card score was not used in the clinical evaluation of volunteers and induration of PP was purely subjective on the part of the study doctor. Training on the psychometric components of the study was received from Dr. Christine Bundy, Senior Lecturer in Behavioural Medicine, based at the University of Manchester.

2.1.2.3 Inclusion and exclusion criteria

Subjects meeting all the following criteria were enrolled in this study:

1. Males or females, aged 18 years and above at the time of consent;
2. Subjects who were able to understand and voluntarily sign an informed consent document prior to any study assessments/procedures being conducted;
3. Subjects who have had a diagnosis of psoriasis by a Consultant Dermatologist or GP, while first signs developed ≤12 months before diagnosis.

The *exclusion criteria* of this study referred to subjects unable to provide written, informed consent and those who had developed their first psoriasis plaques >1 year prior to diagnosis.

2.1.2.4 Study questionnaire

A psoriasis clinical assessment form (the study questionnaire) was designed (**Appendix A**). Upon obtaining written, informed consent the study questionnaire was handed to the study participants to help assess the status of their disease. The questionnaire comprised two parts.

2.1.2.4.1 The clinical section of the questionnaire

The information recorded in this section was:

1. Demographic characteristics
2. Social history
3. Medical history
4. Drug history
5. Family history
6. Triggering factors
7. Clinical pattern of the subject and affected relatives' psoriasis
8. Disease Severity
9. Nail psoriasis
10. Physical observations

The clinical part was completed by ET, apart from medical history which was completed by the patients. Photographs of skin and nails were taken with a CANON EOS 45D digital single-lens reflex (SLR) camera, 12.2 megapixels sensor, with a 18-55 mm IS lens (CANON, UK).

2.1.2.4.2 Demographic characteristics

Information on patients' ethnicity, gender, age, age of onset of psoriasis and disease duration was recorded on the study proforma.

2.1.2.4.3 Social history

Patients were asked about their marital status and occupation. Smoking habits were recorded as the number of cigarettes per day and alcohol consumption as number of units of alcohol per week.

2.1.2.4.4 Medical history

Patients were asked to complete a health assessment proforma of the study questionnaire, which included a number of different diagnoses; **Appendix A**. Patients were asked to tick "yes" on only diagnosed conditions and specify the year of diagnosis. They were also asked to state whether they felt that their non-psoriasis conditions were related to their psoriasis. Some of the diagnoses were linked to a further set of questions, on the back of the health assessment proforma. For example, if a patients ticked "yes" on item 27 (malignancies), then they were prompted to complete the type of diagnosed cancer on the back of the proforma. The same applied for patients with diagnosed depression/anxiety disorders. These patients were asked to identify the

causal event which triggered their mood disorder (family death, divorce or separation, moving house, changing jobs and redundancy). In addition, patients were asked about their allergy profile.

Finally, if patients had been diagnosed with PsA by a Rheumatologist, they were asked to specify their PsA treatment.

2.1.2.4.5 Drug history

Drug history included information on the current and past treatment of psoriasis, as well as the non-psoriasis regularly prescribed medications. Efficacy of previous treatments was assessed and the answer included three different responses; “poor”, “moderate” and “good”. “Poor” efficacy indicated either a minimal or no response to the particular agent or a quick rebound (<3 months). “Moderate” efficacy represented a good response to the agent with moderate improvement of the patient’s symptoms. “Good” efficacy indicated a complete clearance or visually minimal psoriasis. The non-psoriasis medications were cross-checked against the patient’s medical history, to confirm diagnosis.

2.1.2.4.6 Family history

A pedigree chart was designed to include family history of psoriasis and associated comorbidities. The pedigree included relatives from both 1st and 2nd generations. In addition, gender and age of onset of psoriasis in both the study participants and affected relatives were recorded.

2.1.2.4.7 Triggering factors

The factors which contributed to psoriasis flares and/or remission were also recorded in the study questionnaire. Patients were also asked whether exacerbations of their psoriasis were linked to URTIs.

2.1.2.4.8 Clinical phenotype and disease severity

A physical examination was performed to assess disease severity, with the use of PASI (Fredriksson and Pettersson, 1978). In addition, patients were asked to score the overall severity of their disease, over the past week, on a 11-point (0-10) numeric rating scale (patient global psoriasis assessment; PGPA); (Cauli et al., 2011). Information on the clinical phenotypes of psoriasis (chronic plaque, guttate, pustular, PPP, seborrhoeic, scalp, intertriginous and erythrodermic psoriasis) was recorded in the study questionnaire (**Appendix A)**. Plaques were also drawn on a mannequin. The clinical phenotype of affected relatives, when available was recorded. A particular focus was on the clinical pattern of plaque psoriasis. Patients with plaque psoriasis

were, therefore, further classified into large thin, large thick, small thin and small thick patterns and this information was recorded on the study questionnaire (Griffiths et al., 2007b). In addition, photographs of patients' psoriasis were obtained, to confirm the pattern of the disease.

Finally, during the physical examination, baseline observations were recorded, including body mass index (BMI; kilograms/height in meters^2-kg/m^2), blood pressure (BP) and waist circumference.

2.1.2.4.9 Psoriasis Area and Severity Index

The PASI is the current gold standard for assessing the clinical severity and extent of psoriasis (Fredriksson and Pettersson, 1978; Naldi, 2010). It is widely used in randomised controlled trials (RCT) and sometimes in clinical practices to assess the average erythema, induration and scaling of plaques, on a 5-point scale (0-4); (Feldman and Krueger, 2005); **Table 2.1**. The PASI is usually completed by the clinician or a trained nurse. The score is obtained by grading three variables (erythema, infiltration and desquamation) from 0 to 4, weighted by the area of involvement. Each body region is weighted according to its approximate percentage of the whole body, based on the "rule of nines" (Berkow, 1924). The area covered by psoriasis on each body area is estimated as a percentage of the total area of a particular body region. The buttocks are assessed as part of the lower limbs, while the axillae and groin as part of the trunk. The neck is assessed as part of the head. The final score ranges from 0 to 72. Scores below 10 represent mild to moderate disease, from 10-20 moderate to severe disease, while scores over 20 very severe cases of psoriasis.

Body region %TBSA	Erythema	Plaque induration	Scaling	% body surface area with psoriasis
Head and neck **9% of TBSA**	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-** 0% **1-** 1-9% **2-** 10-29% **3-** 30-49% **4-** 50-69% **5-** 70-89% **6-** 90-100%
Trunk, axillae and groin **37% of TBSA**	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-** 0% **1-** 1-9% **2-** 10-29% **3-** 30-49% **4-** 50-69% **5-** 70-89% **6-** 90-100%
Upper limbs **18% of TBSA**	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-** 0% **1-** 1-9% **2-** 10-29% **3-** 30-49% **4-** 50-69% **5-** 70-89% **6-** 90-100%
Lower limbs and buttocks **36% of TBSA**	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-**none **1-**slight **2-**moderate **3-**severe **4-**very severe	**0-** 0% **1-** 1-9% **2-**10-29% **3-** 30-49% **4-** 50-69% **5-** 70-89% **6-** 90-100%

Table 2.1 The parameters of the Psoriasis Area and Severity Index (PASI).

The % of total body surface area (TBSA) refers to adult skin, based on the "rule of nines" (Berkow, 1924).

Component score	Description
Erythema	
0 No involvement	None; may have residual hyperpigmentation
1 Slight	Pink or light red
2 Moderate	Darker pink-red
3 Severe	Red
4 Very severe	Extremely red (“beefy” red)
Induration	
0 No involvement	None
1 Slight	Minimal elevation relative to normal surrounding skin
2 Moderate	Easily palpable with rounded edges
3 Severe	Elevated with hard, sharp borders
4 Very severe	Very elevated, with very hard, sharp borders
Scaling	
0 No involvement	None
1 Slight	Mainly fine scale, some lesions may be partially covered
2 Moderate	Coarser thin scale, most lesions partially covered
3 Severe	Coarser thick scale, nearly all lesions covered, rough
4 Very severe	Very thick scale, all lesions covered, very rough

Table 2.2 Morphologic descriptors for each component of the Psoriasis Area and Severity Index (PASI).

2.1.2.4.10 Patient Global Psoriasis Assessment

Patient global psoriasis assessment is a static measure of disease activity and psychosocial impact of the disease as well as treatment side effects on the day of the clinical visit and is widely used in RCT. Notwithstanding, there is a lack of studies on the clinimetrics of PGPA (Spuls et al., 2010).

The PGPA is usually completed by the patient on either a 0-100 mm visual analogue scale (VAS) or a 0-10 numeric rating scale. Patient’s global assessment of PsA is similar to PGPA measurement and has been validated recently by the American College of Rheumatology (ACR). It is formulated as follows (Cauli et al., 2011; reviewed in Mease, 2011):

“Considering all the ways your arthritis affects you, please mark a vertical line on the scale below (for a VAS scale) your condition, to show how you are feeling today.”

In this thesis, we used a 0-10 numeric PGPA using the following wording:

“Considering all the ways your psoriasis affects you, please grade from 0 (very good-no symptoms) to 10 (very poor-severe symptoms) your condition, to show how you are feeling today.”

2.1.2.4.11 Assessing nail changes

Finger and toe nails were examined for nail psoriasis. The total number of affected nails was completed in the study questionnaire, whilst, as shown in **Table 2.3,** nail changes were assessed on fingernails only and recorded by circling the appropriate signs on the questionnaire.

Nail matrix changes	Nail bed (hyponychium) changes
Pitting	Onycholysis
Leukonychia	Subungual hyperkeratosis
Red spots on the lunula	Oil drop discolouration ("salmon patch")
Nail plate crumbling	Splinter haemorrhage
Beau lines or transverse grooves	

Table 2.3 Nail changes in psoriasis.

The table summarises the most common nail changes seen in patients with psoriasis and/or psoriatic arthritis (PsA). Nail changes are divided in changes of the nail matrix (nail plate) and the nail bed (hyponychium). Onycholysis is the separation of the nail plate from the nail bed. Oil drop discolouration ("salmon patch") refers to a reddish-brown discolouration under the nail plate. Pitting consists of small defined depressions in the nail surface. Nail crumbling is the fragmentation of the friable nail plate, with longitudinal striations (transverse grooves) of the nail plate surface. Leukonychia is represented as white spots in the nail plate, while splinter haemorrhages are small areas of bleeding under the nail, analogous to the Auspitz's sign seen in the skin. Subungual hyperkeratosis refers to the abnormal thickening of the skin of the nail bed. Red spots on the lunula are small pinkish or reddish spots on the lunula. Table adopted from (Baran, 2010).

2.1.2.5 The psychometric sections of the questionnaire

The second part of the questionnaire was completed by the patient and included four, psychometric questionnaires. These were used to evaluate the psychological impact of psoriasis on a subject's daily life (Dermatology Life Quality Index; DLQI), as well as identify changes in mood (Hospital Anxiety and Depression scale; HADS, Penn state Worry questionnaire; PSWQ and Beck Depression Inventory; BDI-II).

2.1.2.5.1 Dermatology Life Quality Index

The DLQI is a general dermatology questionnaire consisting of 10 items and is designed to assess health-related quality of life (QoL) over the previous week (Finlay and Kelly, 1987). The DLQI is extensively used in clinical practice, as well epidemiological and clinical research of

dermatological conditions and has high reliability and internal consistency (Chamian and Krueger, 2004). These 10 questions cover 6 domains of health status: symptoms (1 item) and feelings (1 item), daily activities (2 items), leisure (2 items), work or school (1 item), relationships (2 items) and side effects from therapeutic management (1 item); **Table 2.4**. Each question is graded on a 0 (not affected) to 3 (greatly affected) Likert scale.

The sum of the scores of each of these 10 questions is added up to give a final score which ranges from 0-30. Lower scores represent a better quality of life, while the opposite occurs with high scores; **Table 2.5**. A change of 5 points in the total score indicates a minimal clinically important difference (Chamian and Krueger, 2004).

DLQI domains of health status	Number of items	Maximum score
Symptoms and feelings	Questions 1 and 2	6
Daily activities	Questions 3 and 4	6
Leisure	Questions 5 and 6	6
Work and school	Question 7*	3
Personal relationships	Questions 8 and 9	6
Treatment	Question 10	3

Table 2.4 The Dermatology Life Quality Index (DLQI) analysis.
This table summarises the 6 functional domains covered by the DLQI. Unanswered questions are scored as 0. If there are two or more unanswered questions, then the questionnaire is not scored.
**If "yes", question 7 is scored as 3; if "no" or "not relevant", but then either "a lot" or " a little" is ticked, it is then scored 2 or 1 respectively*.

Total DLQI Score	Meaning of DLQI score
0-1	No effect on subject's QoL
2-5	Small effect on subject's QoL
6-10	Moderate effect on subject's QoL
11-20	Very large effect on subject's QoL
21-30	Extremely large effect on subject's QoL

Table 2.5 Interpretation of the Dermatology Life Quality Index (DLQI) score.
**QoL= quality of life*

2.1.2.5.2 Hospital Anxiety and Depression Scale

The HADS is a 14-item self-administered psychometric questionnaire, used to detect mood disorders (Snaith, 2003; Zigmond and Snaith, 1983). In particular, it is used to identify clinical cases of anxiety and depression, in non-psychiatric hospital patients (Bjelland et al., 2002). Questions on the anxiety subscale (HADS-A) refer to restlessness, worry and panic attacks, while the depression subscale (HADS-D) includes questions on loss of interest or pleasure on normal activities, cognitive impairment and psychomotor retardation; **Table 2.6**. The answers are scored

from 0-3 on a Likert scale, indicating the level of agreement with each item. The questions are divided in two subscales (anxiety and depression) and the total score for each subscale ranges from 0-21. A cut-off score of 8/21 or above, on the anxiety or depression subscales is suggestive of clinical levels of mood disorder, with a sensitivity of 0.9 and a specificity of 0.78 for HADS-A and a specificity of 0.79 and a sensitivity of 0.83 for HADS-D; (Snaith, 2003; Bjelland et al., 2002). Scores between 8 and 10 indicate borderline, whilst scores equal to or higher than 11 on either HADS-A or HADS-D represent clinical cases of anxiety or depression, respectively, which require acute management of the patient.

HADS-A items	HADS-D items
I feel tense or wound up	I still enjoy the things I used to enjoy
I get a sort of frightened feeling as if something bad is about to happen	I can laugh and see the funny side of things
Worrying thoughts go through my mind	I feel cheerful
I can sit at ease and feel relaxed	I feel as if I am slowed down
I get a sort of frightened feeling like butterflies in the stomach	I have lost interest in my appearance
I feel restless and have to be on the move	I look forward with enjoyment to things
I get sudden feelings of panic	I can enjoy a good book or radio or TV program

Table 2.6 The items on Hospital Anxiety and Depression Scale (HADS).
The table summarises the items of the anxiety (HADS-A) and depression (HADS-D) subscales of the HADS questionnaire.

2.1.2.5.3 Beck Depression Inventory-II

The BD-II (Beck et al., 1996b; Beck et al., 1996a) is a 21-item self-completed psychometric measurement and it was designed to assess severity of already diagnosed or suspected depression in adolescents and adults. The BDI-II is a 1996 revision of the original BDI and includes new items based on the updated guidelines for depression, issued by the American Psychiatric Association in the Diagnostic and Statistical Manual of Mental Disorders; fourth edition (DSM-IV); (American Psychiatric Association, 2000). In this study, subjects who scored equal or over 8 in HADS-D were also asked to complete the BDI-II. Items are scored on a 4-point Likert scale (0-3), in increasing severity and its score represent the intensity of a particular symptom of depression, over the previous two weeks. Some items have more than one answer with the same score. The BDI-II is divided in two subscales to cover the two major components of depression; the somatic and cognitive/ affective component of attitudes; **Table 2.7**. All items' scores are summed to provide the total BDI-II score. A total score below 10 indicates minimal depression, while scores

between 10 and 18, and 19 and 29 represent mild and moderate depression, respectively (Beck et al., 1988; Kotila et al., 1998). Finally, scores over 30 are markers of severe depression (Beck et al., 1988; Kotila et al., 1998). The cut-off is 13 and the maximum total score is 63.

BDI-II Subscale Affective component	BDI-II Subscale Somatic component
Pessimism	Sadness
Past failures	Loss of pleasure
Guilty feelings	Crying
Punishment feelings	Agitation
Self-dislike	Loss of interest
Self-criticalness	Indecisiveness
Suicidal thoughts or wishes	Loss of energy
Worthlessness	Change in sleep patterns
	Irritability
	Change in appetite
	Concentration difficulties
	Tiredness and/or fatigue
	Loss of interest in sexual activity

Table 2.7 The main depression components reflected by the Beck Depression Inventory-II (BDI-II).

2.1.2.5.4 Penn State Worry Questionnaire

The PSWQ is a 16-item self-reporting questionnaire which is used, in clinical and non-clinical populations, to detect generalised anxiety disorder (GAD). Generalised anxiety disorder presents with uncontrollable, pathological worry. The PSWQ explores all three main dimensions of pathological worry; generality, excessiveness and uncontrollability (Fresco et al., 2003). Answers range from 1 (not typical of me) to 5 (very typical of me) on a 1-5 Likert scale. Items 1,3, 8, 10 and 11 are reverse scored; if circled “5” on the sheet the actual score is “1”, if circled “2” on the sheet the actual score is “4” and so on. Total scores rank form 16 to 80 (Meyer et al., 1990). The PSWQ performs differently across different populations. Patients with social phobia, for example, score higher on PWSQ compared to normal controls. A score between 45 and 55 is a reference of moderate raised anxiety (within variable clinical presentation), while a score of 65 and above is a definite diagnosis for GAD (Behar et al., 2003; Fresco et al., 2003). **Table 2.8** summarises the mean score of PSWQ with a high specificity and sensitivity, to detect pathological anxiety across different clinical and non-clinical populations.

Type of comparison	N	Mean	SD (±)
Panic disorder	97	53.80	14.76
Panic disorder with agoraphobia	64	58.30	13.65
Generalized anxiety disorder	50	68.11	9.59
Social phobia	54	53.99	15.05
Simple phobia	21	46.90	16.99
Obsessive-compulsive disorder	24	60.84	14.55
Normal controls	32	34.90	10.98

Table 2.8 Validation of the Penn State Worry Questionnaire (PSWQ) across different populations.

The table depicts the mean score and standard deviation (SD) of PSWQ between non-anxious and anxious populations. A score between 45 and 55 is considered clinical levels of moderate anxiety, whereas the general anxiety disorder is captured by a mean score of 68. The table was adopted from (Meyer et al., 1990; Brown et al., 1992).

2.1.3 The retrospective group

The retrospective cohort comprised of psoriasis patients who have attended the weekly psoriasis clinic between the years of 2005-2010, at SRFT. It is standard practice in the SRFT psoriasis clinic, that newly referred patients are clerked using the Salford psoriasis assessment proforma (SPAP). This proforma is completed by the trained staff (dermatology nurses, specialist registrars or clinical fellows) and is very similar to the 1st part of the study questionnaire (**Appendix A**). The proforma includes information on:

- Demographics
- Age of onset of psoriasis
- Social History
- Medical History
- Drug History of anti- and non-psoriasis treatments
- Family history of psoriasis via a pedigree tree where the gender and degree of relationship (1st degree and 2nd degree) of affected member are recorded. In contrast with the study proforma, information on the age of onset of affected relatives is not recorded.
- Nail psoriasis

- PASI and PGPA
- Clinical phenotype of psoriasis but not the clinical pattern of plaque psoriasis (large-thick, large thin, small thin or small thick)

In addition, patients also completed a DLQI as part of their outpatient clerking. Both DLQI and the SPAP are kept in the Dermatopharmacology Unit. An audit approval was placed at the SRFT Audits Department to allow use of the completed proformas. Similar to patients from the prospective cohort, patients from the retrospective cohort were grouped into EOP and LOP patients.

2.1.4 The control group

A control group was used to facilitate the comparison of the prevalence of specific comorbidities (T2DM, IHD and autoimmune thyroiditis-AIT) between EOP and LOP. The control group included patients aged 50 years or above, who were age-matched to the ≥50 years of age, psoriasis patients of the dataset. The controls were randomly selected from patients attending the Minor Injury Unit of the Accident and Emergency (A+E) Department and the Trauma Assessment Unit (TAU), at SRFT, between 2010 and 2012, and were discharged within 12 hours. These patients complained of minor health issues such as respiratory or gastrointestinal infections, dyspepsia, musculoskeletal pain secondary to accidental fall, minor lacerations or fractures after road traffic collisions and non-cardiac, atypical chest pain. Patients' records were accessed via the Salford implemented iSOFT's clinical management software and information on their medical history and BMI was collected from their admission clerking proforma, their referral letters and Salford Integrated Records (SIR). The audit approval for the retrospective collection of data on psoriasis patients was extended to include the controls.

2.1.4.1 iSOFT and Salford Integrated Records

Salford implemented iSOFT's clinical management software is a centralised electronic patients' record system which develops a lifelong single patient electronic record for SRFT patients which is easily and safely shared with other health care providers across UK (Computer Sciences Corporation Healthcare Group, 2012). The SIR is incorporated in iSOFT and details information on patients' health from the Primary and Secondary Care. It is regularly used in the A+E department and allows the physician to quickly assess the patient's medical history, regular prescription, observations and previous referrals (Salford Primary Care Trust, 2012).

2.1.5 Statistical analysis

Statistical analysis was carried out, using IBM SPSS statistics software, version 20·0 for Windows (SPSS Inc., Armonk, NY: IBM Corp U.S.A.). A multivariable analysis was performed to identify significant clinical and psychological differences between EOP and LOP patients. More detailed information is provided in **section 3.3.13** (**Chapter 3**).

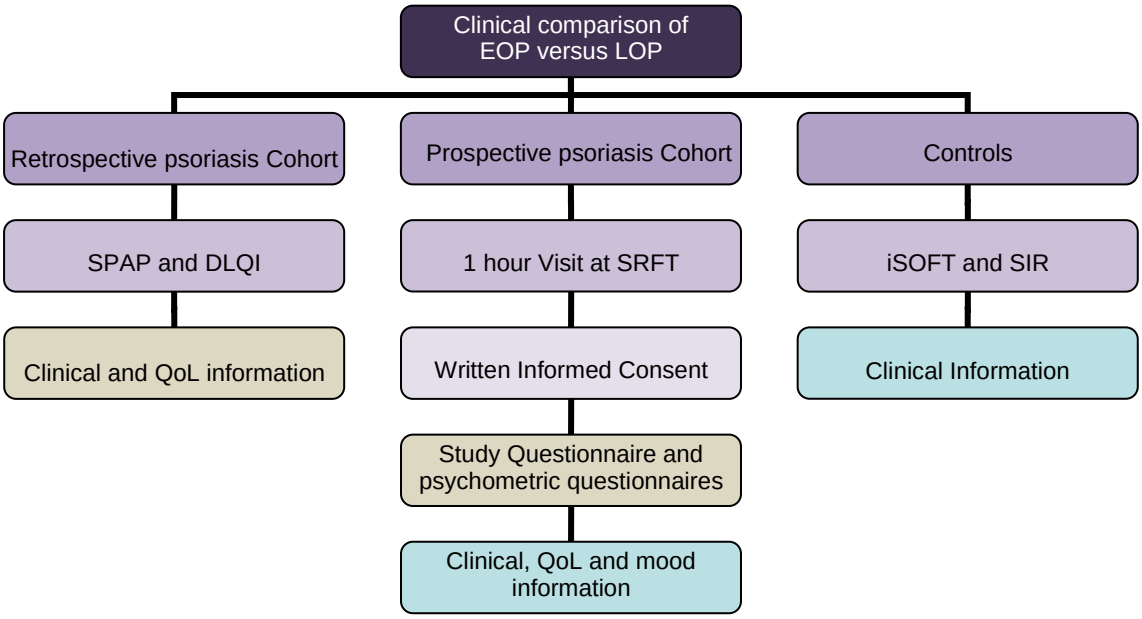

Figure 2.1 Diagram of the questionnaire study design.

EOP=early onset psoriasis, LOP=late onset psoriasis, SPAP= Salford psoriasis Assessment Proforma, DLQI=Dermatology Life Quality Index, QoL=quality of life, SRFT=Salford Royal NHS Foundation Trust and SIR= Salford Integrated Records.

2.2 Histological and immunohistochemical evaluation of early and late onset psoriasis

2.2.1 Study design

A histological study was carried out to examine for potential differences in PN and PP skin, from EOP and LOP patients **(Figure 2.2)**.

2.2.2 Study population

Psoriasis patients were recruited from the weekly psoriasis clinic, Dermatology ward and Phototherapy Unit, at SRFT. Written informed consent was obtained from all volunteers, prior to any study procedures. To limit any confounding effects of age, all enrolled subjects were aged 50 years or above, at the time of recruitment. Enrolled patients were categorised in two groups, based on the age of onset of their psoriasis; the EOP group (age of onset≤ 40 years of age) and the LOP group (age of onset≥ 50 years of age).

2.2.2.1 Ethical approval and study specific training

The study used the ethics of a general biobanking project, "the Skin Procurement study", conducted in the Dermatopharmacology department and was approved by the Salford and Trafford REC (*REC reference number: 05/Q1404/249*). A ten year ethics approval, called the "Immunogenetics of psoriasis", was gained on the 10th of November 2011, one element of which allowed skin biopsy samples to be obtained for this study, (*REC reference number: 10/H1005777);* **Appendix B**. The relevant information leaflets and consent forms were used to obtain written informed consent by all enrolled volunteers.

2.2.2.2 Study visits

Enrolled patients were asked to attend two visits at the Dermatopharmacology clinic, at SRFT. The first was a screening visit, where patients signed the informed consent and eligibility was assessed. The second was the main study visit, where the patients' eligibility was confirmed and then patients had 4 biopsies taken from upper buttock or lower back skin. In keeping with the ICH-GCP guidelines, potential participants were given a verbal description of the study and an information leaflet via post or email, prior to screening. Participants were given at least 24 hours to read through and consider taking part in the study.

All study procedures took place in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2.2.3 Screening visit

Prior to any study procedure, written informed consent was obtained from all subjects; a copy of the consent was given to the subject. Baseline patient information was obtained, including date of birth, sex, race, standard medical history, current medications and treatments, information on psoriasis and PsA specific history (age of onset of psoriasis / PsA, triggering factors and current treatments in the last month). Patients were then asked not to use their medications for a "washout" period, and were scheduled for a skin biopsy, after the “wash-out” (**Table 2.9**).

2.2.2.4 Main study visit

During visit 2, inclusion and exclusion criteria were again reviewed and once the subjects’ eligibility was confirmed, a PASI was completed and photographs of the skin were also taken. The same camera which was used in the questionnaire study, was also used in this study. Afterwards, a skin biopsy was performed.

Finally, blood samples were also obtained the study participants.

2.2.2.5 Inclusion and exclusion criteria

The inclusion and exclusion criteria are presented in detail below. Enrolled patients satisfied all of the inclusion and none of the exclusion criteria.

Inclusion Criteria

- Subjects capable of providing a signed and dated informed consent document, prior to any study related procedures
- Caucasian, adult male and female subjects, aged at least 50 years of age at the time of consent
- Diagnosis of plaque psoriasis by a Consultant Dermatologist or GP, at least six months prior to the study visit (patients with concurrent PsA were eligible for enrollment)
- Subjects with psoriasis on unexposed to sun, upper buttock or lower back
- Subjects receiving topical, systemic treatment or phototherapy were enrolled only if they agreed to have an appropriate drug wash out period (**Table 2.9**).

Exclusion criteria

Potential subjects who met any of the following criteria, were excluded from participating in the study:

- Subjects with non-plaque forms of psoriasis
- Subjects using topical treatment, but unwilling to stop the treatment on lower back and buttock area for at least 2 weeks prior to the study visit

- Subjects receiving any phototherapy, systemic or biologic treatments that may improve psoriasis within the period specified in **Table 2.9**
- Subjects with drug-induced psoriasis
- Subjects receiving lithium, antimalarials, or intramuscular gold within four weeks of the study visit
- Subjects diagnosed with HIV, hepatitis B (HBV), or hepatitis C virus (HCV)
- Subjects diagnosed with uncontrolled renal, hepatic, hematological, pulmonary, cardiac, neurologic, cerebral, or psychiatric disease
- Subjects with other inflammatory skin conditions at the time of consent
- Subjects with a hypersensitivity to lignocaine
- Subjects unable or unwilling to undergo injection of local anaesthetic or to have sutures
- Subjects participating in other studies using investigational agents, procedures, or medical devices during their participation in this study.
- Subjects donating a skin sample which is positive to Periodic acid Schiff (PAS) staining.

Prohibited Treatments	Discontinuation prior to study visit
1. Phototherapy or exposure to sunlight (i.e. tanning booths, sunbathing)	4 weeks
2. Medicated topical treatments (i.e. corticosteroids, vitamin D analogues, retinoids, salicylic acid) on the lower back and buttock area	2 weeks
3. Systemic therapy for psoriasis and PsA (i.e. methotrexate, cyclosporine, fumarates, oral retinoids)	4 weeks
4. Biological agents (e.g. adalimumab, ustekinumab, etanarcept, infliximab)	12 weeks
5. Other investigational psoriasis drugs	4 weeks or 5 half-lives whichever is longer

Table 2.9 Prohibited treatments and duration of washout.

The table summarises the wash-out period from any anti-psoriasis treatment prior to the main study visit. Use of bland emollients was permitted on the lower back and/or buttock area during wash-out. Topical use of any emollients, on lower back and buttock area, was stopped 24hours prior to the main study visit.

PsA=psoriatic arthritis.

2.2.3 Punch biopsy protocol

Patients were asked to lie on their stomach. Their upper buttock skin was cleaned with an anti-septic cleansing solution containing 0.015% mass per volume (w/v) chlorhexidine gluconate and cetrimide 0.15% w/v (STERETS Tipset, Medlock Medical Ltd, UK). Subsequently, anaesthesia was induced at the selected biopsy locations, using lignocaine hydrochloride 1% (Antigen Pharmaceuticals, UK), until the volunteer felt numbness, instead of pain, after a middle-prick on the injected site. Using a 6mm size punch biopsy tool, each volunteer had two pairs of 6 mm, full-

thickness, skin biopsies, taken from sun-protected, upper-buttock or lower back skin. One pair of skin biopsies was from PP and the second pair from PN skin. The minimum distance between PP and PN was 5 cm. Skin biopsies from PP were taken from the edge of the evolving plaques, as this site represents the most active area (Cameron et al., 2002; Griffiths and Barker, 2007a). The resultant wound was sutured with two, 4.0 ethilon sutures (ETHICON, Johnson and Johnson Medical Limited, UK) and dressed with Mepore sterile dressings (Molnlycke Health Care, Sweden). Patients were given a NHS information leaflet on wound care and verbal advice on how to treat their wound and were prompted to book an appointment with their local GP to have the sutures removed 7-10 days later. An ethically approved letter was sent to each participant's GP to notify them about their patient's participation in this study.

A 6 mm PN and a 6 mm PP biopsy were placed in yellow-capped prefilled specimen pots containing 60ml of 10% of neutral buffered formaldehyde (Genta Medical, UK) and were sent to the Cellular Pathology department, at SRFT, for routine H&E staining. The specimen pots were labeled, including the subjects' initials, the visit date and an extra coding method (subject 1, 2, 3, etc), to ensure the anonymisation of data. In addition to the H&E staining, skin sections from the paraffin embedded tissue were processed and stained with IHC staining methods. The two remaining skin biopsies were bisected. One half was snap frozen in liquid nitrogen and then stored at -80^{o}C for future Ribonucleic acid (RNA) work. The other half was first placed in optimal cutting temperature (OCT) embedding medium (Tissue-Tek® OCT ™ Compound, Sakura Finetek Europe B.V., Netherlands), then immediately snap-frozen in liquid nitrogen and stored at -80^{o} C.

The OCT embedding medium is a mixture of water-soluble glycols and resins, which is used to embed tissue specimens prior to snap freezing. The OCT compound helps to eliminate any background staining by blocking the formation of any residues on slides. Using this method, tissue can be stored and processed in the future for IHC. In this thesis, the purpose of having OCT and paraffin embedded tissue, both stored for IHC was that these different media help detect a variety of monoclonal antibodies.

All H&E and IHC slides were reviewed by ET. Dr Lynne Jamieson, Consultant Dermatopathologist, at SRFT, provided training on the appropriate histological and IHC evaluation of slides.

2.2.4 Histological assessment

A histological comparison of PP and PN skin from EOP and LOP patients was performed to explore potential differences in morphology and inflammatory infiltrate between the two subtypes of psoriasis.

2.2.4.1 Haematoxylin and eosin and periodic-acid-Schiff protocol

At the Cellular Pathology department, SRFT, the skin biopsies were first fixed and embedded in paraffin and then stained with H&E. Following tissue cutting, the specimens were

processed on the Tissue Tek VIP 5 processing machine (Bayer, Germany), in which they were fixed in formalin solution, dehydrated through a series of different concentrations of alcohol and cleared in xylene solution, which removes all the alcohol. The tissue sections were then held in molten paraffin wax and then embedded in paraffin wax moulds, using the Leica EG1160 embedding centre (Leica Biosystems, UK). After fixation, the wax-embedded tissue was cut into 3 micrometres(μm) thick sections, with the use of a microtome (Thermo Shandon Ltd, Cheshire). The sections were then floated onto a heated water-bath and picked up onto a slide. Finally, tissue de-waxing with a dewaxing solution and staining with PAS and Haematoxylin (Harris Formulation, Surgipath, Leica Biosystems, UK) and eosin (Surgipath, Leica Biosystems, UK) was performed using the Leica XL Programmable Slide Autostainer (model ST5010, Leica Biosystems, UK). The slides were analysed under a LEICA DM2000 light microscope (Leica Microsystems, UK). For the purpose of this study, 6 levels of H&E sections were placed on each slide and 2 levels of PAS on a second slide. Slides were dried in a 60^0 C tissue drying oven, overnight.

2.2.4.1.1 Haematoxylin & Eosin

Haematoxylin and eosin stains are widely used by pathologists. Haematoxylin has a deep blue-purple colour and stains nuclei, whilst eosin is pink and stains proteins, such as the cytoplasm and extracellular matrix. Cell or tissue structures that take up haematoxylin are commonly called basophilic, while those that take up eosin are called eosinophilic structures.

Haematoxylin alone is also used as a counterstain for IHC procedures which contain alkaline phosphatase or peroxidise (Fischer et al., 2008).

2.2.4.1.2 Periodic acid Schiff

Periodic acid Schiff is widely used to stain glycogen, mucin, mucoprotein, and glycoproteins magenta. It is used to detect fungal infections in skin tissues, as it colours the carbohydrate component of the fungal cell membrane (positive PAS).

2.2.5 Psoriasis Histological Assessment Score

The histological examination of PP and PN skin, from EOP and LOP subjects, was performed, using a modified version of the Trozak histological grading system (**Appendix B**); (Trozak, 1994; Morsy et al., 2010). The Trozak score is used to compare H&E stained skin samples with a histological diagnosis of psoriasiform dermatitis. It comprises 10 different histomorphological features, each taking a score of 1, 2 or 3, depending on their histological specificity for psoriasis and relevance to disease activity (**Appendix B**). Findings such as elongated rete ridges and perivascular dermal oedema, which, according to the author, are not specific for psoriasis, are given a score of 1. On the other hand, thinning of the suprapapillary plate and the presence of Munro microabscesses and / or Kogoj pustules, which are more specific for

psoriasis, take a value of 3. Scores from the 10 variables are then added up to give a total score ranging from 0-19 (**Table 2.11**).

In addition to the 10 features used to calculate a total score, Trozak added two additional histological features to further confirm the histological likelihood of psoriasis: the epidermal thickness (Eth) and mitotic index. The Eth is defined as the average distance in mm, between the base of stratum corneum and the tip of rete ridges, measured in 6 different locations, while the mitotic index is the proportion of keratinocytes undergoing mitosis above the basal layer and is recorded as an average per 8 high power fields (HPF).

To identify histological differences between EOP and LOP samples, a scoring system, essentially based on the Trozak histological assessment, was used, with the addition of 10 histological features and the omission of the presence of suprabasal mitosis and the mitotic index (**Table 2.11**). From these 10 additional features, acanthosis, epidermal/dermal spongiosis, dilated and tortuous papillary blood vessels, epidermal non-lymphoid ICMCs and epidermal/dermal lymphocytes were included in the total score, while epidermal/dermal eosinophils, dermal mast cells and positive/negative PAS stain were not.

The way the findings in this scoring system were assessed, was based on the presence (and severity) of features rather than their relevance to psoriasis, where a score of 0 is awarded when a feature is absent and 3 refers to maximum severity (**Table 2.10**) Subsequently this led to a total histological score (THS), given by adding together the given score for each parameter. The THS ranged from 0-39. In addition to a THS, the histological parameters were further divided into inflammatory and morphological and were respectively used to calculate an inflammatory (total inflammatory score-TIS) and morphological sub-score (total morphological score-TMS); (**Table 2.11**).

In brief, the examined histological parameters were:

Morphological features

1. Extent of loss of the granular layer (hypogranulosis)
2. Epidermal parakeratosis
3. Acanthosis
4. Elongation of rete ridges
5. Thinning of the suprapapillary plate
6. Epidermal spongiosis
7. Dermal papillary oedema
8. Tortuous papillary dermal blood vessels

Cellular elements of inflammation

9. Epidermal neutrophils
10. Epidermal lymphocytes
11. Epidermal eosinophils
12. Epidermal non-lymphoid ICMCs
13. Dermal neutrophils
14. Dermal lymphocytes

15. Dermal eosinophils
16. Dermal mast cells

Epidermal thickness of PP and PN sections was recorded in a similar fashion as in Trozak's worksheet. In addition, a total epidermal thickness (TEth) was measured by subtracting Eth of PN skin from that of PP skin. Epidermal thickness was measured using a standard ocular micrometer (Model 813438, Bausch and Lomb, UK).

Histological Variables	Scoring	Subjective Quantification
Parakeratosis	0-Normal	None across the epidermis
	1-Minimal	Focal parakeratosis across the epidermis
	2-Moderate	Patchy parakeratosis across the epidermis
	3-Severe	Diffuse parakeratosis across the epidermis
Hypogranulosis	0-Normal	None across the epidermis
	1-Minimal	Focal hypogranulosis across the epidermis
	2-Moderate	Patchy hypogranulosis across the epidermis
	3-Severe	Diffuse hypogranulosis across the epidermis
Acanthosis	0-Normal	8-10 epidermal cell layers of the Malpighian layer
	1-Minimal	11-15 epidermal cell layers of the Malpighian layer
	2-Moderate	16-20 epidermal cell layers of the Malpighian layer
	3-Severe	>20 epidermal cell layers of the Malpighian layer
Elongation of rete ridges	0-Normal	None across the epidermis
	1-Minimal	Focal elongation across the epidermis
	2-Moderate	Patchy elongation across the epidermis
	3-Severe	Diffuse elongation across the epidermis
Thinning of the suprapapillary plate	0-Normal	None across the epidermis
	1-Minimal	Focal thinning across the epidermis
	2-Moderate	Patchy across the epidermis
	3-Severe	Diffuse across the epidermis
Epidermal/dermal spongiosis	0-Normal	None across the epidermis/dermis
	1-Minimal	Focal spongiosis across the epidermis/dermis
	2-Moderate	Patchy spongiosis across the epidermis/dermis
	3-Severe	Diffuse spongiosis across the epidermis/dermis
Dermal papillary oedema	0-Normal	None across the dermis
	1-Minimal	Focal oedema across the dermis
	2-Moderate	Patchy oedema across the dermis
	3-Severe	Diffuse oedema across the dermis
Dilated and tortuous papillary blood vessels	0-Normal	None across the upper dermis
	1-Minimal	Small focal areas of dilated and tortuous dermal vessels
	2-Moderate	Several foci of dilated and tortuous dermal vessels
	3-Severe	Multifocal to diffuse dilation and tortuosity of dermal vessels
Epidermal non-lymphoid ICMC	0-Normal	None across the epidermis
	1-Minimal	Focal accumulation of ICMC across the epidermis
	2-Moderate	Patchy accumulation of ICMC across the epidermis
	3-Severe	Diffuse accumulation of ICMC across the epidermis
Epidermal neutrophils	0-Normal	No neutrophils across the epidermis
	1-Mild	Focal epidermal infiltration/focal presence of Munro microabscesses
		Moderate epidermal infiltration/patchy Munro microabscesses
	2-Moderate	Diffuse epidermal infiltration/prominent presence of Munro
	3-Severe	microabscesses and/or presence of Kogoj pustules
Dermal neutrophils	0-Normal	Occasional dermal infiltration
	1-Mild	Focal dermal infiltration
	2-Moderate	Patchy dermal infiltration
	3-Severe	Diffuse dermal infiltration
Epidermal/dermal lymphocytes	0-Normal	Occasional epidermal/dermal infiltration
	1-Mild	Focal epidermal/dermal infiltration
	2-Moderate	Patchy epidermal/dermal infiltration
	3-Severe	Diffuse epidermal/dermal infiltration
Epidermal/dermal eosinophils	0-Normal	No infiltration across the epidermis/dermis
	1-Mild	Focal epidermal/dermal infiltration
	2-Moderate	Patchy epidermal/dermal infiltration
	3-Severe	Diffuse epidermal/dermal infiltration
Dermal mast cells	0-Normal	Occasional infiltration across the dermis
	1-Mild	Focal dermal infiltration
	2-Moderate	Patchy dermal infiltration
	3-Severe	Diffuse dermal infiltration

Table 2.10 The semi-quantitative scoring system for the histological evaluation of skin samples.

The table displays the histological parameters, as well as the 4 point semi-quantitative scale which was used to assess the involved (PP) and uninvolved (PN) skin sections. "Minimal" refers to the presence of 1-2 foci of the examined feature or affects <10% of the examined area, "focal" represents 3-4 foci or 10-25% of the examined area, "moderate" refers to 5-8 foci or 26-50% of the examined area and "severe" is >8 foci or affects >50% of the examined area.

**ICMC= intraepidermal collections of mononuclear cells*

Microscopic Criteria	Trozak grading system	Psoriasis Histological Assessment Score
Parakeratosis	1-Focal 2-Diffuse	0-Normal 1-Focal 2-Patchy 3-Diffuse
Hypogranulosis	1-Focal 2-Diffuse	0-Normal 1-Focal 2-Patchy 3-Diffuse
Acanthosis	Not recorded	0-Normal 1-Focal 2-Patchy 3-Diffuse
Elongation of rete ridges	1- Regular elongation 2-"Club-shaped" rete ridges	0-Normal 1-Focal 2-Patchy 3-Diffuse
Thinning of the suprapapillary plate	2- Presence of suprapapillary plate thinning	0-Normal 1-Focal 2-Patchy 3-Diffuse
Epidermal spongiosis	Not recorded	0-Normal 1-Focal 2-Patchy 3-Diffuse
Mitosis above the basal layer	2-presence of suprabasal mitosis and Calculation of the mitotic index	Not recorded
Dermal papillary oedema	1-presence of elongated and oedematous dermal papillae	0-Normal 1-Focal 2-Patchy 3-Diffuse
Dilated and tortuous papillary blood vessels	Not recorded	0-Normal 1-Small focal areas 2-Several foci 3-Multifocal to diffuse
Epidermal non-lymphoid ICMC	Not recorded	0-Normal 1-Focal infiltration 2-Patchy infiltration 3-Diffuse infiltration
Epidermal neutrophils	3-Presence of Monro microabscesses* and 3-Presence of Kogoj pustules*	0-Normal 1-Focal presence of Munro microabscesses 2-Patchy Munro microabscesses 3-Prominent Munro and/or presence of Kogoj microabscesses
Dermal neutrophils	1-presence of perivascular mononuclear infiltrate in the upper dermis of papillae	0-Normal 1-Focal infiltration 2-Patchy infiltration 3-Diffuse infiltration
Epidermal/dermal lymphocytes	Not recorded	0-Normal 1-Focal infiltration 2-Patchy infiltration 3-Diffuse infiltration
Total histological score **Total morphological score** **Total inflammatory score**	**0-19** Not measured Not measured	**0-39, THS=TMS+TIS** **0-24** **0-15**
Epidermal/dermal eosinophils	Not recorded	0-Normal 1-Focal infiltration 2-Patchy infiltration 3-Diffuse infiltration
Dermal mast cells	Not recorded	0-Normal 1-Focal infiltration 2-Patchy infiltration 3-Diffuse infiltration
Epidermal thickness	Average distance from the base of stratum corneum to the tip of rete ridges in 6 locations for PP skin	**TEth= Eth of PP - Eth of PN**

Table 2.11 Comparison between the Trozak and the study grading system (psoriasis histological assessment score).

*TEth=Total epidermal thickness, PP=involved skin from psoriasis patients, PN=uninvolved skin from psoriasis patients, ICMC= intraepidermal collections of mononuclear cells, THS=total histological score, TIS=total inflammatory score, TMS= total morphological score. * These parameters are assessed separately in the Trozak score, while in this thesis score, these are encountered as different severity levels of the same parameter (epidermal neutrophils)*

2.2.6 Immunohistochemical assessment

Following the H&E examination of slides, an IHC comparison of PP and PN tissue sections from EOP and LOP patients was performed to explore further differences in the inflammatory component of these two subtypes of psoriasis. This comparison was carried out using IHC antibodies against specific lymphocytic (CD3, CD4 and CD8) and non-lymphocytic (CD1α) markers.

2.2.6.1 Immunohistochemical staining

A Leica Bond-Max fully automated staining system (Leica Microsystems, UK) was used to stain the tissue sections (Leica Microsystems, UK). Primary antibodies for CD3, CD4, CD8 and CD1α were purchased by Leica Microsystems (UK) and DAKO, (Denmark); (**Table 2.12**). The various reagents which were used include;

a) Bond Polymer Refine Detection system (DS9800, Leica Microsystems, UK), which contains a peroxide block, a post primary immunoglobulin (Ig) G linker, a polymer horseradish peroxidase (HRP) reagent, 3,3'-diaminobenzidine (DAB) tetrahydrochloride, used as chromogen, and haematoxylin, and which was used to block the endogenous peroxidase activity, bind and visualise the IHC antibodies.

b) Bond™ Dewax Solution (AR922, Leica Microsystems, UK), for dewaxing formalin-fixed paraffin-embedded samples.

c) Bond™ Wash Solution 10x concentrate (AR9590, Leica Microsystems, UK), which is a concentrated buffer solution (Tris buffered saline-TBS, surfactant and 3.5% ProClin™ 950), used to remove excess reagent from the tissue section, before the new reagent was added.

d) Bond™ DAB Enhancer (AR9432, Leica Microsystems, UK) which is a heavy-metal solution and increases the contrast between the DAB-specific staining and the slide background.

Antigen retrieval was performed prior to staining, to break the methylene bridges (formed during fixation) and hence, expose the antigenic sites. Antigen retrieval was performed using heat mediated epitope retrieval technique for 30 minutes (heat induced epitope retrieval-HIER) in with a citrate based buffer and surfactant adjusted to pH 8.0 (Bond Epitope Retrieval Solution 1- AR9961 and solution 2- AR9640, Leica Microsystems, UK).

Five μm thick, paraffin-embedded tissue sections were placed on charged slides and these were loaded in the Leica Bond-Max stainer. Tissue-dewaxing was performed at 72^0C, for 10 minutes, with the Leica Microsystems' Dewax solution and sections were then washed at ambient temperature. To reduce any non-specific background staining, the activity of endogenous peroxidase was blocked using 3–4% volume per volume (v/v) hydrogen peroxide for 10 minutes. The slides were then washed 3 times, with the Bond™ Wash Solution. Sequentially, the pre-diluted primary antibody was added and slides were incubated for 30 minutes and this step was followed by a three washes. A post-primary linker [post primary (30 mL) Rabbit anti-mouse IgG in 10% v/v animal serum, in TBS/0.09% ProClin™ 950] was added for 10 minutes, to localise the primary antibody. Again three washes followed to remove the excess reagent and then a polymer secondary antibody (polymer anti-rabbit poly-HRP-IgG, containing 10% v/v animal serum in TBS/0.09% ProClin™ 950) was added for 10 minutes, to reduce background staining. In a similar fashion, two washes with the Bond™ Wash Solution took place, to remove the excess reagent and these were followed by washes with distilled water for another 4 minutes. The substrate chromogen (DAB Part 1 or 66 mM 3,3'-DAB tetrahydrochloride, in a stabiliser solution and DAB Part 2 or ≤0.1% v/v hydrogen peroxide, in a stabiliser solution), was added and slides were incubated for 10 minutes. Diaminobenzidine was catalysed to its oxidised form by hydrogen peroxide, resulting in a dark-brown precipitate. A series of washes with distilled water followed before the final step; haematoxlin was then added as a counterstain and slides were incubated for another 5 minutes. Finally, the slides were washed with distilled water and dried overnight.

Table 2.12 summarises the primary antibodies used in this study and their optimised concentrations. Stained cells (positive cells) yielded a brown colour. A 5 μm section from tonsils was used as positive control tissue. Negative control sections were provided for each biopsy and these lacked the primary antibody.

Tissue sections of PP and PN skin were randomised and the assessor (ET) was blinded. The slides were reviewed under a Leica DM2000 light microscope (Leica Biosystems, UK), at 200X magnification. Positive epidermal cells were counted per microscopic field at 200X magnification. More specifically, positive epidermal cells were counted from the stratum corneum down to the basement membrane, across the whole section and the absolute number was divided by the number of microscopic fields. The dermal infiltrate was assessed using a semi-quantitative (0-3) scale from the basement membrane to lower dermis, also across the whole section. Absolute epidermal $CD3^+$, $CD4^+$, $CD8^+$ and $CD1\alpha^+$ counts and epidermal $CD4^+/CD8^+$ ratios were calculated.

Primary Antibody	Provider	Species/Clones	Reaction Location	Optimised Concentration
$CD3^+$	Leica	Monoclonal Mouse anti-human CD3 cell/ LN10	Membrane and cytoplasm	1:250
$CD4^+$	Leica	Monoclonal Mouse Anti-human CD4 cell/ 4B12	Membrane	1:400
$CD8^+$	Dako	Monoclonal Mouse anti-human CD8 cell/ C8/144B	Membrane and cytoplasm	1:100
$CD1\alpha^+$	Dako	Monoclonal Mouse anti-human CD1α cell/ 010	Membrane and cytoplasm	1:100

Table 2.12 The immunohistochemical antibodies of the biopsy study.

The table shows the different antibodies of the immunohistochemistry (IHC) part of the biopsy study. Information on the supplier from which they were purchased, the species and clone, the location of the reaction and dilution are provided.

2.2.7 Statistical analysis

Numerical data were explored for normality using the Kolmogorov-Smirnov test and were considered normally distributed if P-value (P) >0.05. Homogeneity of variances among the groups was tested using Levene test and equal variances were assumed if P >0.05.

Continuous and discrete numerical data (age, age of onset, disease duration, PASI, TEth, THS, TMS, TIS, absolute counts of $CD3^+$, $CD8^+$, $CD4^+$, $CD1\alpha^+$ and the $CD4^+/CD8^+$ ratio) were analysed with descriptive statistics and are presented in the thesis as mean or median and standard deviation (SD).

Ordinal and nominal categorical variables (histological variables) are presented as frequencies and percentages.

Independent samples t-test or *analysis of variance (ANOVA)* were applied to compare means of normally distributed continuous numerical data or Mann-Whitney U test for non-normally distributed numerical data and the independent samples median test for discrete numerical data. This first level of analysis did not account for covariates. The second level of analysis include a linear regression model to examine for links between histological parameters and the two subtypes of psoriasis (EOP vs LOP) and adjust for confounders. For the histology study, three outcome variables were employed and these were the THS, TMS and TIS. The main independent variable was a binary (0 or 1) one, with 0 representing EOP and 1 LOP. For the IHC study, the outcome variables included the absolute counts of $CD3^+$, $CD4^+$, $CD8^+$ and $CD1\alpha^+$ cells. The confounders for both analyses (histological and IHC) included disease duration, PASI, clinical phenotype and gender. Significance values were indicated as significant, if * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

IBM SPSS statistics, version 20·0 software for Windows (SPSS Inc., Armonk, NY: IBM Corp U.S.A.) was employed for the statistical analysis.

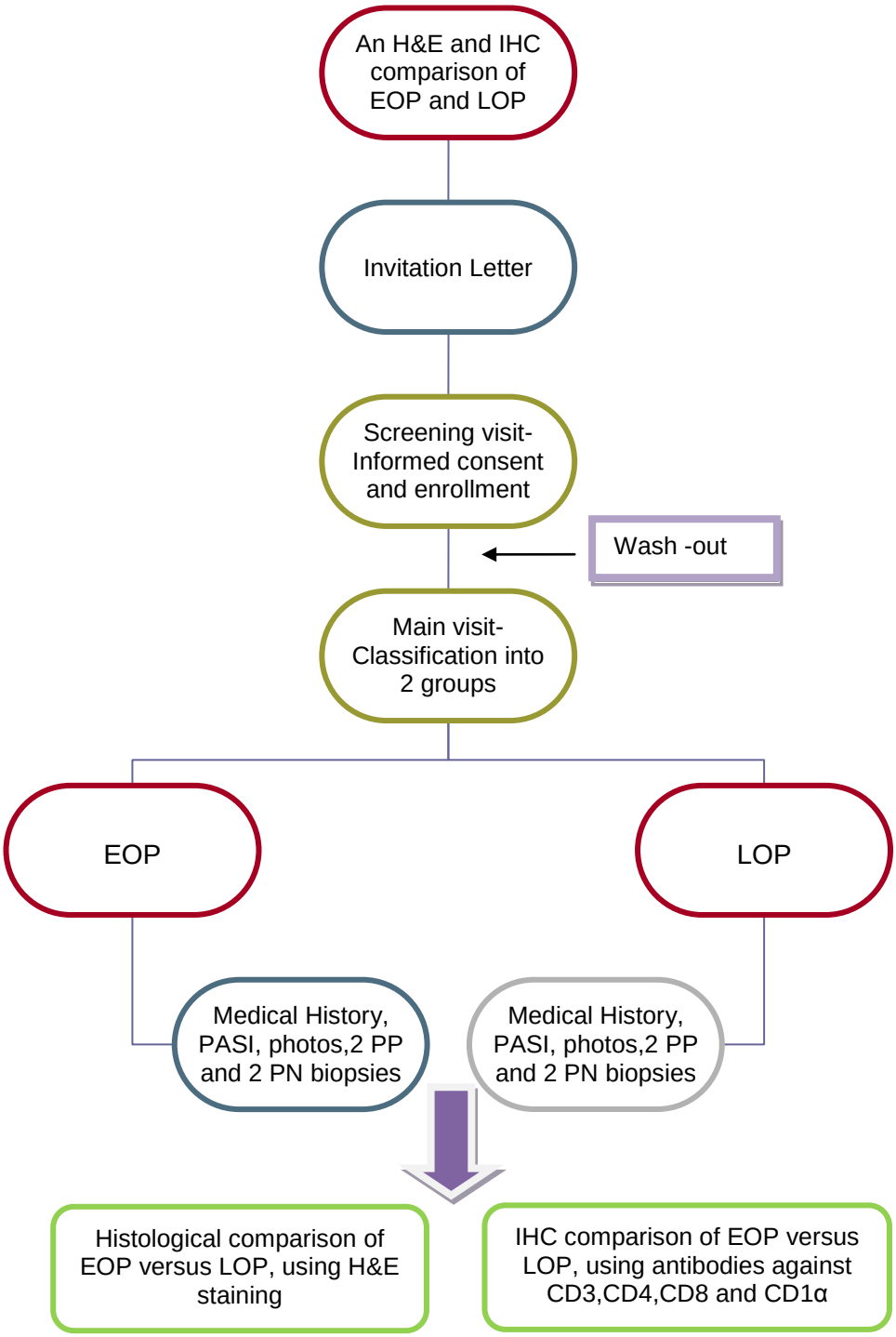

Figure 2.2 Flow chart of the biopsy study design.

H&E; haematoxylin and eosin, IHC; immunohistochemistry, EOP; early onset psoriasis, LOP; late onset psoriasis, PASI; psoriasis Area and Severity Index, PP; involved skin from patients with psoriasis, PN; uninvolved skin from patients with psoriasis, CD; cluster of differentiation.

3. A clinical investigation of early and late onset psoriasis

3.1 Introduction

It is has been shown previously that EOP and LOP differ in disease severity, anatomical area of involvement, family history of psoriasis, and psychological impact. More specifically, EOP has been associated with more extensive, severe and unstable skin disease, with frequent flares and a high psychosocial burden (Ferrandiz et al., 2002; Henseler and Christophers, 1985; Gupta et al., 1996; Mallbris et al., 2005). There is a strong family history of psoriasis in EOP and the *HLA-Cw*06:02* allele is found in approximately 50-80% of EOP patients (Henseler and Christophers, 1985; Ferrandiz et al., 2002; Allen et al., 2005). Those patients with EOP, who are *HLA-Cw*06:02 positive,* are also more likely to develop a guttate phenotype, post URTIs from group A β-haemolytic streptococcus, whilst they have more frequently suffered from childhood atopic eczema (Mallbris et al., 2005; Weisenseel et al., 2002). Furthermore, *HLA-Cw*06:02* positive patients are also more likely to develop the Koebner phenomenon, following physical trauma of PN skin (Gudjonsson et al., 2006a). In addition, recent research has demonstrated that young psoriasis patients with severe disease have a higher risk for IHD, findings which subsequently associate EOP with major cardiovascular events (Gelfand et al., 2006). Finally, recent findings show that EOP patients frequently report improvement of their psoriasis after sun exposure (heliotherapy), generalised application of mud or clay (pelotherapy), bathing in the sea (thalassotherapy) or immersing in thermomineral water pools (balneotherapy), whilst other studies support that EOP patients are more likely to receive systemic, 3rd line treatments, such as biologic agents (Gudjonsson et al., 2002; Harari et al., 2012; Di Lernia and Ficarelli, 2012).

On the other hand, LOP shows a milder and stable disease course and a relative low psychosocial burden (Ferrandiz et al., 2002; Henseler and Christophers, 1985). Family history of psoriasis is less common, while the *HLA-Cw*06:02* allele occurs approximately in 15% of patients (Henseler and Christophers, 1985), a frequency which is comparable to that found in the general population. To a large extent, *HLA-Cw*06:02* negative patients are reported to have scalp and nail involvement, as well as PsA (Gudjonsson et al., 2006a; Gudjonsson et al., 2002). Interestingly, recent data, which contradict those published by Gelfand and colleagues, reveal that compared to EOP, LOP is linked with T2DM and metabolic syndrome and hence, LOP patients are potentially at a higher risk for IHD (Armesto et al., 2012; Mazlin et al., 2012; Gelfand et al., 2006).

Despite the aforementioned, identified clinical and genetic differences between EOP and LOP, many aspects of this interesting classification are still not clear, such as potential differences in the clinical pattern of psoriasis, the triggering factors and the response to different treatments. In addition, in the face of contradictory evidence from previous studies, there is still more to be found about the association of either EOP or LOP with any comorbidities.

Thus, the aim of this part of the thesis was to conduct an observational clinical cohort to further explore the previously documented clinical differences, whilst investigate for additional differences.

3.2 Objectives

The primary objective of this study was to examine for potential demographic, clinical and psychosocial differences between EOP and LOP patients, in a UK hospital and community based population. Specific secondary aims include:

1. To confirm existing differences that are described in the literature between these two subtypes of psoriasis; for example, relation to family history of psoriasis, clinical severity, psychological disability and ethnic variations.

2. To identify clinical phenotypes specific to either EOP or LOP.

3. To investigate if the association of other medical conditions (T2DM, hypertension-HTN, cardiovascular disease, Crohn's disease, PsA) with psoriasis is specific to either EOP or LOP.

4. To explore differences in the use and response to different treatments.

3.3 Methods

A retrospective observational study was conducted, along with a prospective, observational, single-centre, population-based study, in a geographically defined, multiracial community, to explore possible clinical and psychosocial differences between EOP and LOP. In addition, using an age matched control group of non-psoriasis subjects, differences in the prevalence of psoriasis associated comorbidities were explored, between the two subtypes of psoriasis, as compared to the general population.

3.3.1 Psoriasis prospective arm of the questionnaire

A total of 112 psoriasis patients from Greater Manchester, UK, were enrolled in the prospective study between 2010 and 2012.

3.3.2 Psoriasis retrospective arm of the questionnaire

Data from 239 psoriasis patients were included in this study. These were computerised stored data of patients attending the psoriasis and Phototherapy clinics, SRFT, between 2005 and 2010. Patients were from the Greater Manchester area, UK.

3.3.3 Control group

The control group was comprised of 157 non-psoriasis subjects, aged 50 years or above. Subjects were randomly selected from the electronic patient records system (via access to the Salford implemented iSOFT clinical management software), at SRFT. All subjects were patients who had been discharged within 12 hours of stay at the SRFT. Patients with chronic inflammatory disorders and cancer patients were excluded.

3.3.4 Study cohort and clinical evaluation

The participants of the prospective cohort were asked to attend a one hour visit at the Dermatopharmacology clinic, SRFT. An extensive physical examination, followed by a detailed clinical history, was performed. Clinical information was recorded in a previously designed, structured questionnaire which included date of birth, age of onset of psoriasis, disease duration, presence of related comorbidities, family history of psoriasis and related comorbidities, psoriasis and severity assessment, number of hospital inpatient visits for psoriasis, exacerbations secondary to infection, nail involvement, triggering factors, response to current and previous anti-psoriatic treatments (**chapter 2, section 2.1.2.4**). Changes in the clinical pattern of psoriasis after anti-psoriatic treatment were also recorded in the questionnaire. Patients were recorded as suffering from another medical condition, only if they have been diagnosed and treated for that condition with cross-referencing of the medication prescribed and the diagnosed comorbidities. Clinical examination included assessment of the distribution and size of psoriasis plaques and body site involvement. Nail changes were categorised into nail bed and nail plate changes and recorded in the questionnaire. Psychometric questionnaires were completed by the patients.

Patients were categorised into two groups based on the age of onset of their psoriasis; the EOP group (age of onset ≤40 years) and the LOP group (age of onset ≥50 years). The same classification system was applied to parents of the study participants.

In the retrospective cohort, clinical and demographic data, from newly referred psoriasis patients, were extracted from a structured proforma, similar to the one used in the prospective arm. This proforma was filled out by either a clinician, a clinical or research nurse, during the weekly psoriasis and Phototherapy clinic. Demographic and clinical information of age, gender, age of psoriasis onset, family history of psoriasis, diagnosed comorbidity, nail involvement, therapeutic management and disease severity were recorded on the proforma. Patients were also clinically assessed and PASI and DLQI were obtained.

In the control group, baseline demographic information (age, gender and ethnicity), medical history and clinical observations were obtained (via iSOFT) from their admission clerking proforma at A+E, their clinical notes from previous admissions, their previous referral letters, their SIR and their SIR medication history.

3.3.5 Assessing disease severity

Based on the severity assessment classification used in the 2002 Gudjonsson et al paper, three parameters of disease severity were used; PASI, PGPA and DLQI (Gudjonsson et al., 2002). In addition, anti-psoriasis treatment was considered when classifying patients into mild to moderate, moderate to severe and very severe groups. In particular, patients receiving continuous systemic treatment were categorised in the very severe psoriasis group, those on extensive topical treatment or phototherapy were placed in the moderate to severe group and those on localised topical treatment, in the mild to moderate severity group, irrespective of PASI. For subjects on localised topical treatment, severity was assessed as follows: patients with PASI< 10, DLQI<10 or PGPA≤3 were classified as mild to moderate severity psoriasis, while moderate to severe psoriasis patients were those with PASI between 10 and 15, DLQI between 10 and 15 or PGPA between 4 and 7. The group of very severe psoriasis patients included those with PASI≥15, DLQI≥15 or PGPA≥8 (**Table 3.2**).

3.3.6 Assessing the clinical phenotype

Patients with chronic plaque psoriasis were classified in four different groups based on the clinical pattern of their psoriasis: the small thin plaque psoriasis group (plaque size ≤3cm and induration ≤0.75mm), the small thick plaque group (plaque size ≤3cm and induration >0.75mm), the large thin group (plaque size >3cm and induration ≤0.75mm) and the large thick group (plaque size >3cm and induration >0.75mm); (Griffiths et al., 2007b). This classification was applied in the prospective arm only, as the relevant data were not recorded in the retrospective arm of the study.

Patients from both arms of the study were placed in the following seven groups based on the phenotype of their psoriasis: plaque psoriasis, scalp psoriasis, guttate psoriasis, pustular psoriasis, PPP, intertriginous psoriasis and sebopsoriasis. Patients with drug-induced psoriasis were excluded from the study.

3.3.7 Assessing family history of psoriasis

In the prospective arm of the study, family history of psoriasis was assessed in two different ways: a) patients were asked whether they had a positive family of psoriasis in their 1st (parents, siblings and offspring) or 2nd degree (aunts, uncles, grandparents, grandchildren, nieces, nephews, or half-sibling) relatives, while; b) information of the gender and age of onset of psoriasis of affected relatives were also recorded in the questionnaire.

In the retrospective arm of the study, data included information on family history of psoriasis (yes or no) in the 1^{st} and 2^{nd} degree relatives and the gender of the affected family member, but lacked any information on the age of onset.

Data on the family history of psoriasis, from adopted patients, were not included in the analysis.

3.3.8 Assessing psoriasis-related comorbidities

Patients from both arms of the study were asked whether they had been diagnosed with the following diseases: T2DM, Crohn's disease, PsA, depression, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, asthma, AIT, HTN, dyslipidemia, IHD and cancer. Information on the year of diagnosis and the various medications used to treat the previously mentioned conditions was recorded.

Patients from the prospective arm only, were also asked to recall whether any of their family members ever suffered from the aforementioned comorbidities. Data on the family history of comorbidities, from adopted patients, were not included in the analysis.

In the control group, a particular focus was on recording a diagnosis of T2DM, AIT, HTN, dyslipidemia and IHD.

3.3.9 Assessing triggering factors of psoriasis

Patients from both arms of the study were asked whether stressful events, seasonal changes, URTIs, medications or changes in menstrual cycle (for female patients) exacerbated their psoriasis. Menstrual changes included premenstrual stage, pregnancy and menopause. There was the option for those who experienced flares from other factors to record these triggers in the questionnaire under "other". In addition, patients were asked to recall any factors that improved their psoriasis (medications, sunlight, lifestyle changes and menstrual cycle changes). If patients had experienced any remission of their psoriasis, the date and duration of remission was recorded, as well as potential factors that, according to the patients, may have induced the remission.

3.3.10 Assessing psychological impairment

The impact of psoriasis on daily life was assessed using the total score of the DLQI, which was completed by all psoriasis patients (from the prospective and the retrospective arms); **chapter 2, sections 2.1.2.5.1**. A score between 0-1 indicated "no effect on QoL", a score of 2-5 represented "a small effect on QoL", a score of 6-10 was "a moderate effect on QoL", 11-20 was "a very large effect on QoL" and lastly, a score over 20 indicated "an extremely large effect on QoL".

Mood disorders, including anxiety, worry and depression, were assessed using the HADS, PSWQ and BDI-II (**chapter 2, sections 2.1.2.5.2, 2.1.2.5.3** and **2.1.2.5.4**). These were completed only by patients on the prospective arm of the study. Scores ≥8 on HADS-D were considered clinical levels of depression and these patients were asked to complete BDI-II. Scores of≥45 for PSWQ and ≥8 on the anxiety scale of HADS-A were considered clinically significant anxiety.

3.3.11 Measurement of other covariates

Gender (male or female), ethnicity, marital status and occupation were recorded. Based on the statistical bulletin of the office for National Statistics (Office for National Statistics, 2011), marital

status was encoded in four groups (single, married, widowed and divorced). According to the social grades of the National Readership survey (National Readership Survey, 2008), occupation was initially arranged in six different classes (upper middle class, middle class, lower middle class, skilled working class, working class, lowest level of subsistence) and these were then grouped in two main categories (middle class and working class). The smoking behaviours and alcohol use were assessed on the basis of self-reported number of smoked cigarettes per day and consumed units of alcohol per week respectively. The units of alcohol were calculated based on the alcohol publication of the Department of Health, "Units and You" (Central Office of Information's Publications team, 2008). BMI, waist circumference, systolic and diastolic BP were recorded.

3.3.12 Sample size and study power

Based on the sample size calculations from 2 studies (Ferrandiz et al., 2002; Armesto et al., 2012), for each tested hypothesis, a minimum of 18 subjects per group was required to detect a statistically significant difference between probability proportions (δ) of 15% for each clinical variable, at a 5% significant level (P<0.05) and 90% power (Campbell and Machin, 1999).

$$n = (Z_{\alpha}+Z_{2\beta})^2(\pi 1\ (1-\pi 1) + \pi 2\ (1-\pi 2) / \delta_2$$

π_1= probability proportion of non-exposure in cases
π_2= probability proportion of exposure in cases
δ= difference in proportions (π_2-π_1)
n= sample size
α=significance level (0.05)
1-β= power (≥80%)
Z_{α}, $Z_{2\beta}$= ordinates of the normal distribution

Equation 3.1 Sample size calculation for the comparison of proportions

In addition, the seminal paper of Peduzzi and colleagues suggests that a minimum 10 events (10 subjects) per variable are required, in a multivariate analysis, to avoid type I and type II errors (Peduzzi et al., 1996). Thus, in this study after accounting for the number of variables (in the current model 20), a minimum of 200 patients were required to produce powered results. A total of 351 patients were actually enrolled; 112 prospectively and 239 retrospectively.

3.3.13 Statistical analysis

Data were explored for normality using the Kolmogorov-Smirnov test and were assumed to be normally distributed if P > 0.05. Homogeneity of variances among the groups was tested using Levene test and equal variances were assumed if P >0.05.

Continuous and discrete numerical data (age, age of onset, disease duration, waist circumference, BMI, BP, alcohol consumption, smoking, number of hospital visits, number of nail involvement, disease severity, PASI, DLQI) were analysed with descriptive statistics and are presented as mean, SD and median.

Ordinal and nominal categorical variables (gender, marital status, occupation, family history of psoriasis, comorbidities, family history of comorbidities, anti-psoriatic treatments and response to treatment, clinical phenotype of psoriasis, psoriatic nail changes and triggering factors) are presented as frequencies and percentages. Missing values were not included in the analysis.

BMI was modelled in an ordinal variable (18.5-25 normal BMI, 25.1-30 overweight, >30 obese), based on the BMI classification by the World Health Organisation (WHO) ;(World Health Organisation, 2000).

Alcohol consumption was also transcribed as an ordinal variable, based on the usual alcohol intake per week (low risk or responsible drinking, increased risk or hazardous drinking, higher-risk or harmful drinking); (NICE, 2012). Responsible drinking (low risk for alcohol related disorders) includes regular consumption of <21 units of alcohol per week for adult males or <14 units of alcohol per week for adult females. Hazardous drinking (increased risk for alcohol related disorders) relates to a consumption of alcohol between 22-50 units per week for adult males or 15-35 for adult females. Harmful drinking (higher risk for alcohol related disorders) includes consumption of >50 units per week for adult males or > 35 units per week for adult females.

Smoking behaviour was grouped in 4 categories (≤10 cigarettes per day, 11-20 cigarettes per day, 21-30 cigarettes per day, ≥31 cigarettes per day), based on the classification of the Fagerström Test for Nicotine Dependence (Heatherton et al., 1991).

The analysis was performed in three stages:

The chi-square (x^2) test was employed to identify associations between two categorical variables (2x2 table) or the Fisher exact test, when x^2 test was not applicable (expected cell count >0 but <5). In addition, when the expected count of at least one cell was 0, in a 2x2 table, the test of independent proportions was used, via Statsdirect statistical software (StatsDirect, Ltd, UK). An *independent samples t-test* was applied to compare means of normally distributed continuous numerical data or Mann-Whitney U test for non-normally distributed and discrete numerical data. Analysis of variance was used to compare means among >2 groups. This first level of analysis did not account for covariates. Correlations were assessed with the Pearson's Correlations Coefficient (r) test for normally distributed data and the Spearman's rank Correlation Coefficient (ρ) test. These were particularly employed to identify significant correlations between PASI and DLQI, PASI and PGPA, DLQI and HADS, as well as, DLQI and PSWQ and assess concurrent validity. A Pearson's r or Spearman's ρ between ±0-0.2 indicates "no association", ±0.2-0.4 is a "weak association", ±0.4-0.6 "moderate association", ± 0.6-0.8 shows a "strong association" and ±0.8-1.0 represents a "very strong association".

Binary logistic regression models were used to explore associations and risk factors and adjust for confounders. The main outcome variable was a binary marker (0 or 1) for EOP-0 and LOP-1, for all analyses, apart from the analysis on comorbidities, where the outcome variable (0 or 1) represented the relevant comorbidity (T2DM, AIT, Crohn's disease, PsA, atopy, IHD). Significance values were indicated as significant, if * P <0.05, ** P < 0.01 and *** P < 0.001. A P value <0.1 was chosen as a threshold of significance ("trend towards significance"); (Cox and Snell, 1981).

IBM SPSS statistics software, version 20·0 (SPSS Inc., Armonk, NY: IBM Corp U.S.A) was used to perform the statistical analysis.

3.4 Results

3.4.1 Demographic data of enrolled patients

3.4.1.1 General characteristics

Of the 112 patients in the prospective cohort, 108 (53 female; mean age 52.1±16.2 years and 55 male; mean age 52.5±14.1years) were included in the analysis. Three patients with an age of onset of 42 years, 43 years and 44 years were excluded from the analysis, as they fell into the overlapping onset group of 41-49. In addition, a female LOP subject did not complete the whole of the questionnaire (apart from the basic clinical information), due to emotional distress, when she was faced with the psychometric questionnaires. Her clinical data, including age of onset, gender, ethnicity and family history of psoriasis, were included in the analysis. The vast majority of patients (91%) were white. Demographic data of the study participants are detailed in **Table 3.1**. Seventy-six patients (41 male and 35 female) were included in the EOP group with a mean age of onset of 20.3±9.9 years and a mean disease duration of 25.8±15 years, compared to 32 patients (14 male and 18 female) in the LOP group with a mean age of onset of 55.6±7 years and a mean disease duration of 10.2±7.9 years.

A total of 232 subjects from the retrospective cohort were analysed; 6 patients had an age of onset of their psoriasis between 41-49 years and hence, were excluded from the analysis. The demographic data of the retrospective cohort are detailed in **Table 3.1**. Two hundred and two patients had EOP (mean age of onset 20.7±9.9 and mean disease duration 30.2±13.3 years), while 30 subjects suffered from LOP (mean age of onset 55.2±7.2 and mean disease duration 15±7.1 years).

In total, the data from 340 psoriasis patients were used for the analysis of certain variables (family history of psoriasis in 1st and 2nd degree relatives, diagnosed comorbidities, treatment, disease severity, clinical phenotype and QoL). From the 340 patients, 278 were EOP patients (mean age of onset of 20.6±9.9y and mean disease duration of 28.9±14 years) and 62 LOP patients (mean age of onset of 55.4±7 years and mean disease duration of 12.6±7.8 years). As shown in **Table 3.1**, similar to the data from the prospective study, age of onset of psoriasis was not affected by the gender of the subjects in both the retrospective cohort and the total of 340 patients.

The control group comprised 157 non-psoriasis subjects with a mean age of 66±11.2years. From the 157 subject, 193 (54%) were male and 164 (46%) female. Ninety five percent of controls were white British, whilst the rest were of Asian origin (2% Pakistani and 3% Indian). The controls were age matched to the 198 psoriasis subjects, over the age of 50years, from the prospective and retrospective cohorts. The control group was used in the analysis of the psoriasis associated comorbidities and reflects the prevalence of particular, psoriasis related comorbidities, in the general population.

Demographic Data	Frequencies (%) and Mean ± SD			T-test (P value) for gender differences		
	Prospective (I)	Retrospective (II)	Total (III)			
Ethnic origin						
White	98 (91)	227 (98)	326 (96)			
Other	10 (9)	5 (2)	14 (4)			
Sex						
Male	55 (51)	123 (53)	178 (52)			
Female	53 (49)	109 (47)	162 (48)			
				I	**II**	**III**
Age of subjects	52.3±15.1	53.4±14.9	53.1±15			
Male	52.5±14.1	52.9±14.9	52.8±14.5	-	-	-
Female	52.1±16.2	54.1±15.1	53.4±15.4	*0.91*	*0.55*	*0.68*
Age of onset of psoriasis (years)	30.8±18.6	25.2±15	26.9±16.4			
Male	30.4±16.8	26.3±14.9	27.5±15.6	-	-	-
Female	31.2±20.4	23.9±15.1	26.3±17.3	*0.82*	*0.24*	*0.49*

Table 3.1 Demographic data of the subjects participating in the psoriasis prospective (N=108) and retrospective cohorts (N=232), as well as the total of psoriasis patients (N=340). *This table shows the demographic characteristics of patients from the prospective and retrospective cohorts. In addition, it shows the comparison of means in age and age of onset of psoriasis, between male and female participants of each cohort.*

**SD, standard deviation from the mean*

3.4.1.2 Familial predisposition to psoriasis

A total of 335 patients were analysed (106 from the prospective and 229 from the retrospective study), as 5 patients were adopted (2 from the prospective and 3 from the retrospective study). A hundred and seventy one out of 276 EOP patients (62%) reported a positive family history of psoriasis in their first or second degree relatives, compared to 21 out of 59 LOP patients (35.6%), and this difference was statistically significant, irrespective of subject's gender (*logistic regression; Odds ratio-OR of family history= 0.33, P<0.001, 95% confidence interval-CI 0.18-0.59);* (**Figure 3.1**). The majority of participants with a positive family history of psoriasis, from both groups (EOP and LOP), reported a higher occurrence of familial psoriasis in the 1st degree-relatives; **Table 3.6**.

To further examine whether family history of psoriasis in the different generations plays an important role in EOP and LOP, a binary logistic regression model was employed, in which EOP and LOP acted as a binary dependent variable, whereas family history of psoriasis in the different generations was the independent factor and the gender of subjects was the main covariate. Interestingly, it was found that, compared to patients with no family history, patients with affected first degree relatives were 59% less likely to be LOP *(logistic regression; OR=0.41, P=0.011, 95% CI 0.22-0.86),* while patients with an affected second degree relative, were 70% less likely to

develop LOP *(logistic regression; OR=0.3, P=0.018, 95% CI 0.12-0.89)*. Finally, patients with a strong positive family history of psoriasis in both generations had the lowest probability of suffering from LOP *(logistic regression; OR=0.12, P=0.005, 95% CI 0.03-0.58)*. The previous findings show that positive family history of psoriasis is inversely related to age of onset.

To extend and further elaborate the previous findings, it was explored whether age of onset can be passed down from parents to offspring. Information on the age of onset of psoriasis in affected parents was only available in the prospective data, with a total of 107 cases included in this analysis. The exclusions were 2 EOP patients who did not know the age of onset of psoriasis of their affected parent, 2 EOP patients who were adopted and 1 EOP patient who had an affected parent with an age of onset at 42 years. Twenty two EOP (29% of EOP patients; 19 with an EOP parent and 3 with a LOP parent) and 5 LOP subjects (15.6% of LOP patients; 4 with a LOP parent and 1 with an EOP parent), reported having affected parents with psoriasis. Most notable was that the age of onset of psoriasis in affected parents and offspring were significantly associated *(Fischer's Exact Test; P=0.005)*. Looking at the gender of affected parents, 12 EOP and 3LOP patients had an affected mother with psoriasis, while 10 EOP and 2 LOP patients had an affected father. The gender of affected parents did not correlate with the type of psoriasis of the subjects *(Fisher's Exact Test; P=0.65).* A logistic regression was employed to investigate whether age of onset of psoriasis in the affected parents was an important factor in developing either EOP or LOP. This time, the dependent variable (outcome) was still the binary EOP vs LOP in the study participants, while the type of psoriasis in affected parents (categorical variable with three categories; no affected parents, EOP affected, LOP affected parent) was treated as the independent factor. The gender of the subjects was used as a covariate. The current findings showed that, compared to subjects with no affected parents, patients with EOP parent(s) were 91% less likely to develop LOP *(binary logistic regression; OR=0.093, P=0.025, 95% CI 0.012-0.74).* On the other hand, patients with LOP parent(s), had a fourfold higher likelihood of developing LOP. This, however, was not statistically significant (**Table 3.4**). When the gender of the affect parents was included in the regression model, it was noticed that, although the parental gender did not significantly relate to the outcome, it slightly affected the relationship between the age of onset of affected parents and the outcome *(logistic regression; OR=0.13, P=0.075, 95% CI 0.014-1.23).*

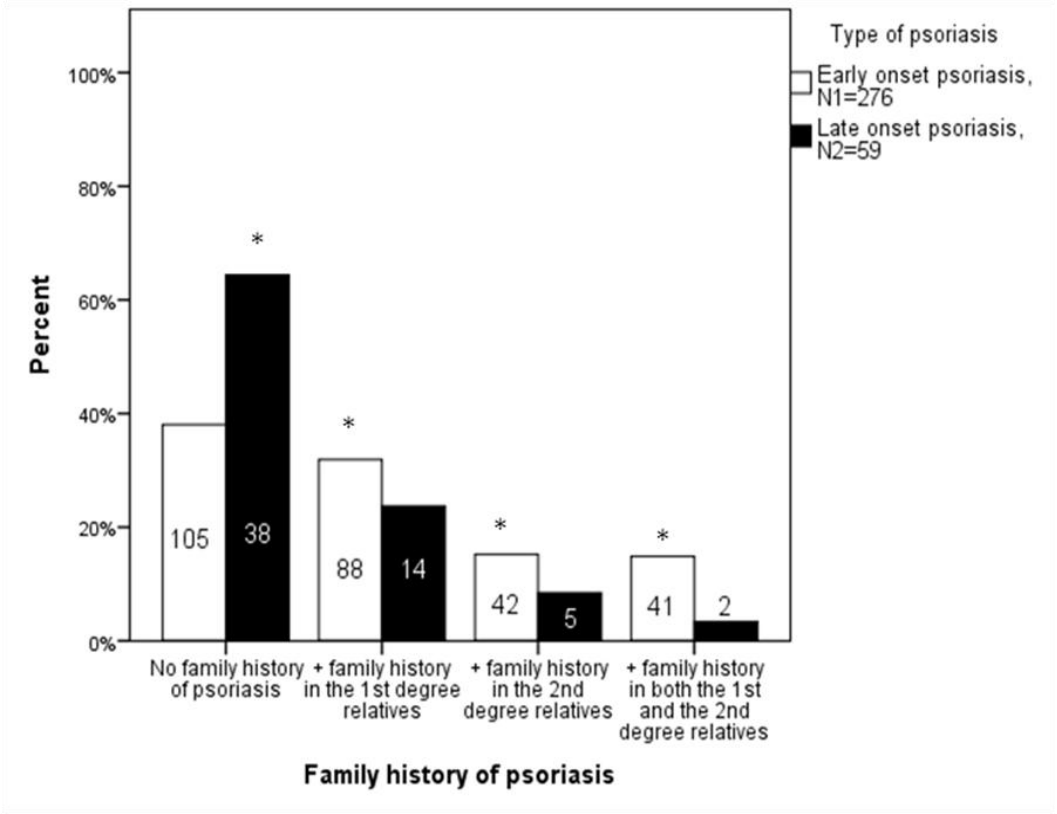

Figure 3.1 Association of the type of psoriasis with family history (N=335).

The bar chart shows the percentage (y axis) and absolute frequencies (in or on the top of the bars) of patients with early (EOP) and late onset psoriasis (LOP), associated with a positive or negative family history for psoriasis. Asterisk (*) represents statistically significant differences (P≤0.05) between the study groups (EOP and LOP), produced after chi square (x^2)-analysis was employed. The graph depicts that EOP patients are more likely to have a positive family history for psoriasis.

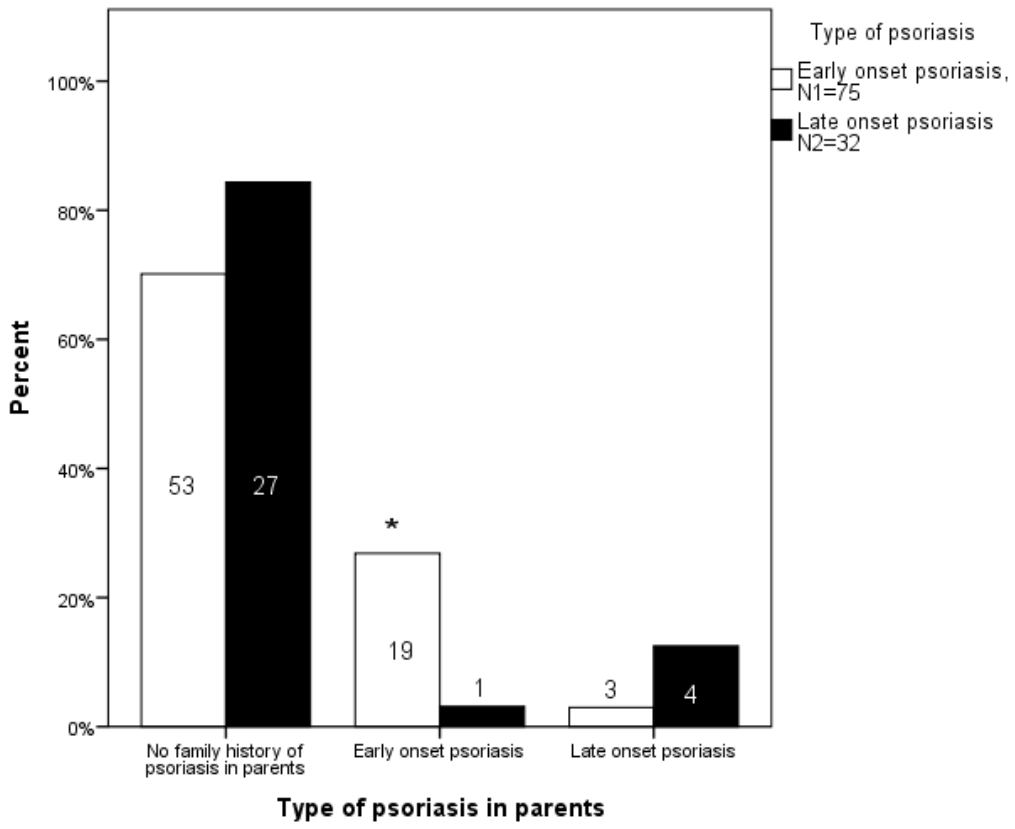

Figure 3.2 Association of the type of psoriasis with parental psoriasis (N=107).

The bar chart shows the percentage (y axis) and absolute frequencies (in or on the top of the bars) of early (EOP) and late onset psoriasis (LOP) patients with parental psoriasis. Asterisk (*) represents statistically significant differences (P≤0.05) between the study groups (EOP and LOP), generated after chi square (x^2)-analysis was applied. The graph demonstrates that EOP patients are more likely to come from EOP parents.

3.4.2 Social characteristics

The social habits of patients were assessed between the two subtypes of psoriasis. In particular, special focus was given to alcohol consumption and smoking status. From the total of 340 patients, it was found that both the EOP and LOP patients were smoking on average 5 cigarettes per day *(t-test; t=0.23, P=0.94)*. In addition, EOP patients had a slightly higher consumption of alcohol per week (EOP; 18 units/week compared to LOP; 10 units/week), but this finding was not statistically significant *(t-test; t=0.77, P=0.48)*.

Marital status was examined in all 340 subjects. It was found that LOP patients were less likely to be single compared to EOP patients *(x^2; P=0.022)*, but when data were stratified by age, including only those aged >50, this finding shifted from significant to non-significant *(x^2; P=0.33)*.

3.4.3 Clinical characteristics

3.4.3.1 Disease severity

Based on the criteria in **Table 3.2**, patients from both psoriasis cohorts were assessed for the severity of their disease. Prior to the analysis of the study data, a Spearman's Correlation test was computed to examine whether the severity parameters are positively correlated. The PASI, PGPA and DLQI were all positively related at a moderate level (Spearman's Correlation; $\rho_{PASI-PGPA}$=0.66, $\rho_{PASI-DLQI}$=0.56 and $\rho_{DLQI-PGPA}$= 0.63, P<0.001 for all correlations). In addition, comparison of means was performed to detect whether PASI, DLQI and PGPA were significantly different between EOP and LOP subjects. In the total of 340 patients, there were no significant differences in neither PASI *(t-test; t=1.22, P=0.23) n*or DLQI *(t-test; t=0.098, P=0.92), nor PGPA (t-test; t=1.893, P=0.22),* between EOP and LOP patients.

However, using the adapted severity score, EOP patients from the prospective study showed a trend towards significance in developing severe disease (x^2*; P=0.067)*, compared to LOP patients. When the retrospective data were included in the analysis, the previous relationship became statistically significant *(x^2; P=0.021)*. Moreover, LOP patients were less likely to have received continuous systemic treatment (18.3%) or biologic agents (3.3%), compared to EOP patients (24% and 15.9% respectively); **Table 3.5**. Finally, there was no difference in the number of hospital inpatient visits for psoriasis between the two subtypes of psoriasis; mean $_{\text{hospital visits for EOP patients}}$=2±7 and mean $_{\text{hospital visits for LOP patients}}$ = 2±5; *t-test, P=0.89.*

Table 3.2 Disease Severity-Study Criteria.

Mild disease	Moderate disease	Severe disease
PASI<10 / PGPA ≤3 *Or* No treatment / localized topical Treatment *Or* DLQI<10	PASI 10-14.9 / PGPA 4-7 *Or* Intermittent systemic treatment/phototherapy/ extensive topical treatment *Or* DLQI 10-14	PASI≥15 / PGPA≥8 *Or* Biologic agents/ Continuous Systemic Treatment *Or* DLQ I≥15

PASI=Psoriasis Area and Severity Index, PGPA=patient global psoriasis assessment, DLQI=Dermatology life quality index

Clinical and Psychological Severity Assessment Tools	EOP Mean ± SD	LOP Mean ± SD	t statistics	P value
PASI	8.3 ± 6.4	7 ± 5.4	1.23	0.23
DLQI	8 ± 8	8 ± 7	0.098	0.92
PGPA	5 ± 3	5 ± 2	1.9	0.22

Table 3.3 Clinical and psychological tools to assess disease severity.

The table demonstrates the individual t-tests performed to detect significant differences in clinical severity and impact in quality of life (QoL) between the two subtypes of psoriasis (early onset psoriasis or EOP versus late onset psoriasis or LOP).

SD= standard deviation, PASI=Psoriasis Area and Severity Index, PGPA=patient global psoriasis assessment, DLQI=Dermatology life quality index

3.4.3.2 Clinical Phenotype

A total of 323 subjects (264 EOP and 59 LOP) were included in this analysis, as we lacked information on 17 patients from the retrospective cohort (14 EOP and 3 LOP). Chronic plaque psoriasis was the most prevalent clinical phenotype; 86.7% of EOP patients and 88.1% of LOP patients. Guttate psoriasis (4.9% of EOP patients), pustular psoriasis (0.8% of EOP patients), and erythrodermic psoriasis (1.9% of EOP patients) were exclusively seen in the EOP group of patients (**Figure 3.3**). Scalp psoriasis and PPP were slightly more frequent in the LOP group (5.1% of LOP patients compared to 1.1% of EOP patients for the scalp psoriasis and 3.4% of LOP vs 1.5% of EOP patients for PPP); **Table 3.4**.

Information from the prospective study (N=84 patients; 61 EOP and 23 LOP), on the clinical pattern of plaque psoriasis (large thick, large thin, small thick and small thin phenotype) demonstrated that large thick plaque psoriasis was the most prevalent form of plaque psoriasis in EOP patients (39.3% of EOP patients), with large thin plaque psoriasis being the second most frequent clinical form of psoriasis (29.5%). The opposite pattern was seen in the LOP patients; 47.8% of LOP patients had large thin plaque psoriasis, while 26.1% presented with large thick plaque psoriasis (**Figure 3.4**). This relationship, although notable, was not statistically significant *(x^2; P=0.115 for the large thin pattern and P=0.207 for the large thick pattern)*. Interestingly, small thick plaque psoriasis was solely seen in EOP patients, but again this was not found to be statistically significant when compared with LOP patients (*test of independent proportions, P=0.334*).

Nail psoriasis was seen in both groups and the number of affected nails did not significantly differ between groups *(t-test; t=1.27, P=0.21)*. Interestingly, when the individual nail changes were assessed, it was found that psoriatic changes of only the nail bed were significantly associated with LOP (10.7% of EOP patients vs 29.5% of LOP patients, *x^2, P=0.015*). A logistic regression model was computed to account for confounders; PsA and gender of subjects. It was

observed that patients with changes of the nail bed (especially onycholysis and hyperkeratosis) had approximately a threefold higher chance in being LOP patients, compared to those with no nail changes *(logistic regression; OR=3.42, P=0.042, 95% CI 1.05-11.23).*

Flares of psoriasis following URTIs were significantly more frequent in the EOP group (28.3% of EOP patients vs 16.1% of LOP patients; x^2, *P=0.049*). By default, flares during pregnancy or postpartum were perhaps unsurprisingly seen solely in EOP patients, while flares during menopause were present in both groups (3% of EOP patients vs 10.5% of LOP patients; x^2, *P=0.29).* Stress was the main exacerbating factor in both groups; 63.2% in EOP patients and 50% of LOP patients. Both groups reported psoriasis free intervals during the course of their disease (38.2% of EOP patients and 29.4% of LOP patients; x^2, *P=0.376*). Those psoriasis free periods were usually induced during treatment or following sunny, relaxing holidays.

Clinical Phenotypes of psoriasis	Frequencies (%) EOP (N=264)	LOP (N=59)	X^2 (P value)
Plaque psoriasis	229 (86.7%)	52 (88.1%)	0.562
Large Thick[1]	24 (39.3%)	6 (26.1%)	0.207
Large Thin[1]	18 (29.5%)	11 (47.8%)	0.115
Small Thick[1]	6 (9.8%)	0 (0%)	0.316[2]
Small Thin[1]	13 (21.3%)	6 (26.1%)	0.641
Guttate psoriasis	13 (4.9%)	0 (0%)	0.082[2]
Scalp psoriasis	3 (1.1%)	3 (5.1%)	0.077[3]
Intertriginous psoriasis	7 (2.7%)	1 (1.7%)	1.000[3]
Palmoplantar psoriasis	4 (1.5%)	2 (3.4%)	0.302[3]
Pustular psoriasis	2 (0.8%)	0 (0%)	0.503[2]
Seborrhoeic psoriasis	1 (0.4%)	1 (1.7%)	0.332[3]
Erythrodermic psoriasis	5 (1.9%)	0 (0%)	0.287[2]
Total	264 (100%)	59 (100%)	

Table 3.4 Clinical phenotypes of psoriasis between the study groups (N=323).

The table shows the distribution of clinical phenotypes of psoriasis between early onset (EOP) and late onset psoriasis (LOP) groups. [1]The analysis includes patients from the prospective arm of the study (N=84) and comparisons are performed with chi-square (x^2). [2]To detect significant differences, when the expected count of cells was 0, the test of independent proportions was used. [3]To detect differences, when the expected count of cells was >0 but <5, the Fischer's exact test was employed.

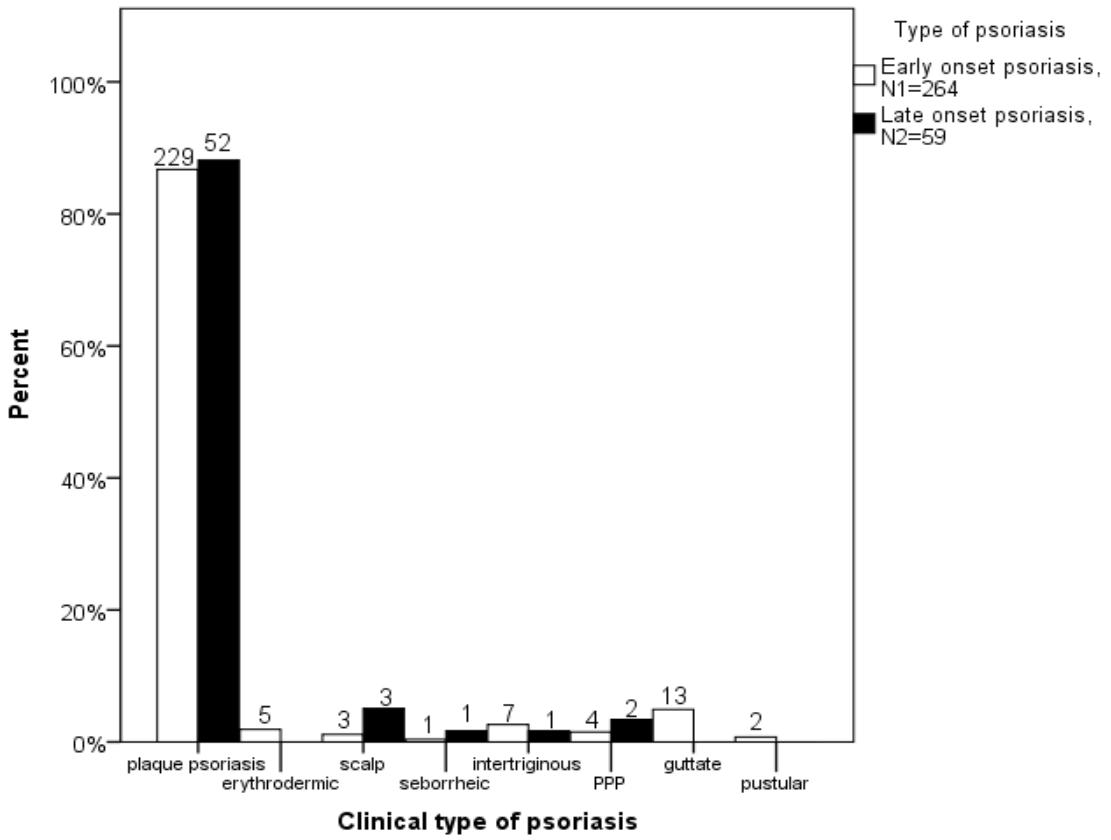

Figure 3.3 Type of psoriasis and frequencies of clinical phenotypes of psoriasis (N=323).

This bar chart shows the percentage (y axis) and absolute frequencies (in or on the top of the bars) of early (EOP) and late onset psoriasis (LOP) patients with different clinical phenotypes of psoriasis.

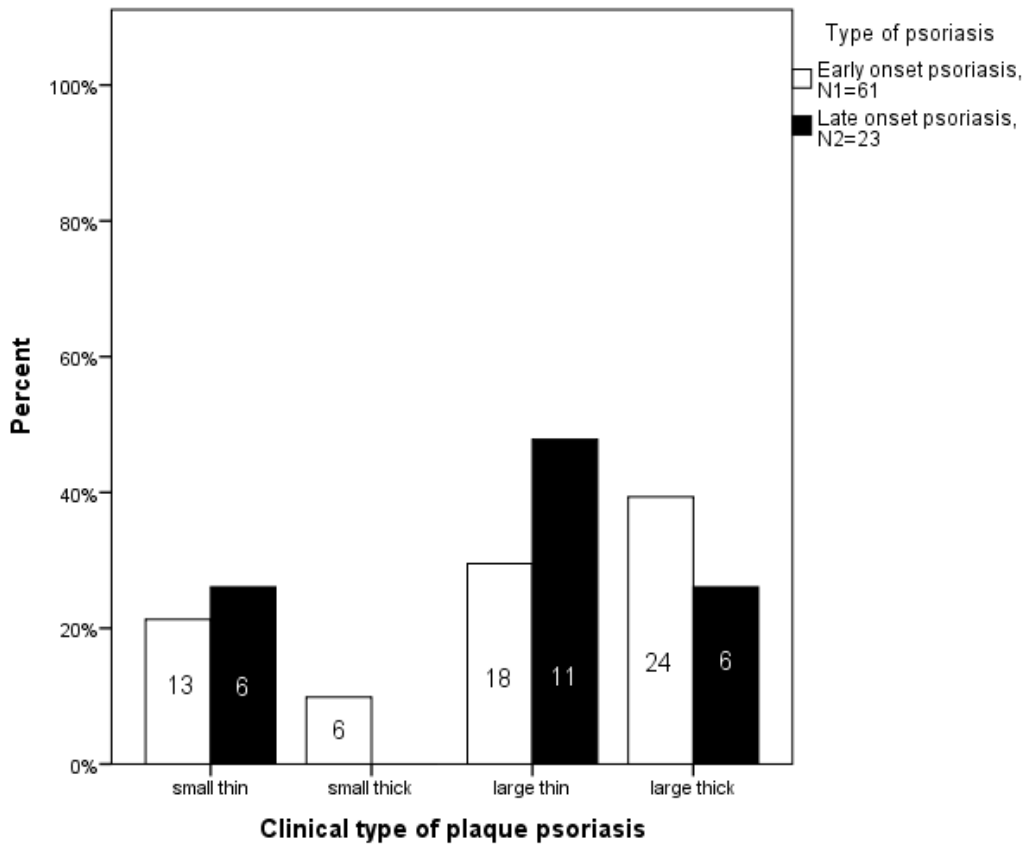

Figure 3.4 Type of psoriasis and frequencies of phenotypes of plaque psoriasis (N=84).
The bar chart demonstrates *the percentage (y axis) and absolute frequencies (in or on the top of the bars) of early (EOP) and late onset psoriasis (LOP) patients with different clinical patterns of plaque psoriasis. The data are obtained from the prospective arm of the study.*

3.4.3.3 Therapeutic management

Different therapeutic agents were assessed between the two study groups. **Table 3.5** shows the current and previous anti-psoriatic treatments used by the study participants of both psoriasis cohorts. Compared to the LOP group, there was a notable higher proportion of EOP patients receiving biologic agents *(x^2; P=0.010);* **Figure 3.5**. On the other hand, LOP patients were more frequently undergoing phototherapy (UVB); *Fisher's Exact Test, P<0.001*. By adjusting for age ≥50 years, the previous findings remained statistically significant *(for biologics, 13.3% of EOP vs 3.3% of LOP patients*; *x^2, P=0.036 and for phototherapy, 13.3% of LOP vs 3.7% of EOP patients; x^2, P=0.013)*

There was no significant difference in reported responses to various previous, different therapeutic agents, but numbers were quite small to compare, as for many agents (fumaric acid esters, retinoic acid and biologic agents), were not applicable for the LOP group.

Anti-psoriatic agents	Current Treatment EOP	LOP	X^2 (P value)	Past Treatment EOP	LOP
No treatment	6 (2.2%)	1 (1.7%)	1.000		
Topical treatment	145 (53.5%)	38 (63.3%)	0.166	55 (72.4%)[1]	20 (58.7%)[1]
Phototherapy	5 (1.8%)	8 (13.3%)	<0.001*[2]	44 (57.9%)[1]	14 (40%)[1]
Systemics	65 (24%)	11 (18.3%)	0.346	20 (26.3%)[1]	7 (20.6%)[1]
Biologics	43 (15.9%)	2 (3.3%)	0.010*		
Systemics and Biologics	7 (2.6%)	0 (0%)	0.358[2]		
	271 (100%)	60 (100%)			
	Total %				

Table 3.5 Current and past anti-psoriasis treatments (N=331).

Data are presented in frequencies and percentages (%). Data from 9 patients (7 EOP and 2 LOP) were missing and were not included in the analysis. [1] The total of these percentages does not add up to 100, as patients may have tried multiple combinations of these treatments in the past. [2]Fischer's Exact test, as the expected cell count of at least one cell in the comparison was <5. Asterisk (*) represents statistically significant differences (P≤0.05).

EOP= early onset psoriasis, LOP=late onset psoriasis, x^2=chi-square

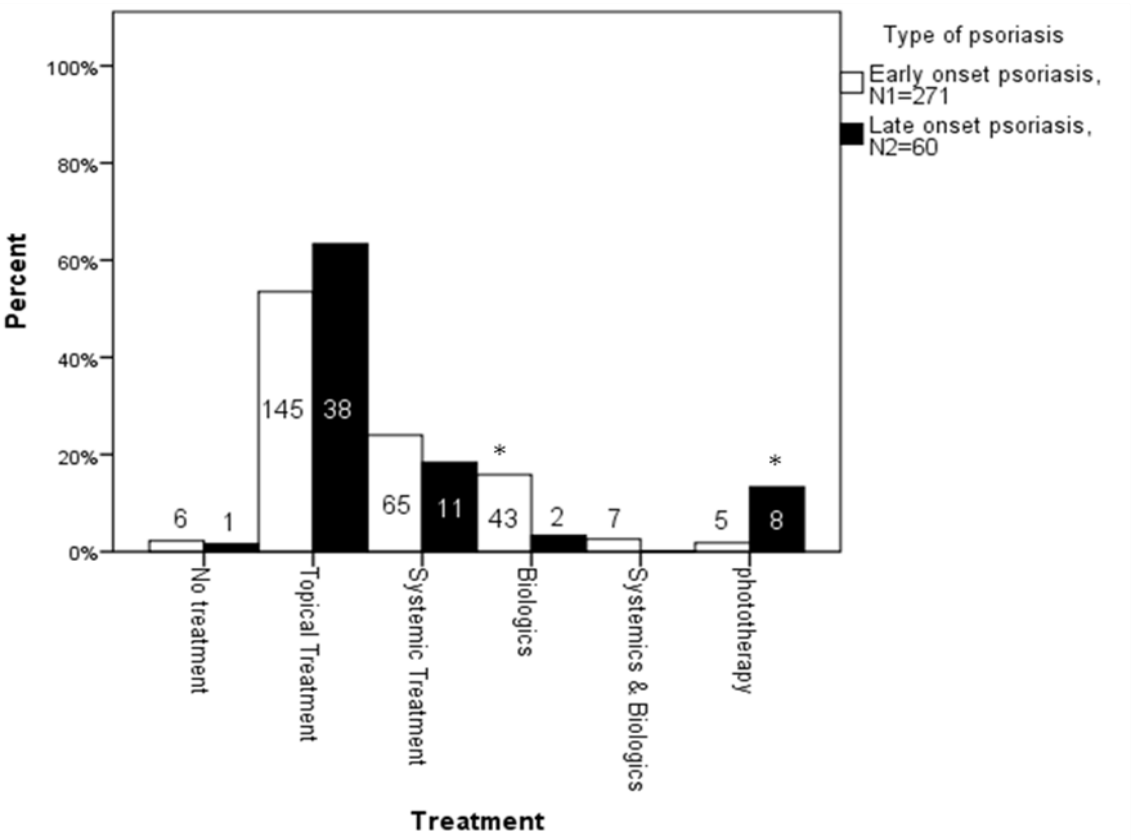

Figure 3.5 Type of psoriasis and therapeutic management (N=331).

The bar chart shows percentage and absolute frequencies of patients with early (EOP) and late onset psoriasis (LOP) using different treatments for their psoriasis. Asterisk (*) represents statistically significant differences (P≤0.05), generated after x^2-analysis was applied. The graph illustrates that EOP patients are more likely to receive biologic agents compared to LOP patient who are more likely to undergo phototherapy.

3.4.4 Associated comorbidities

3.4.4.1 Psoriatic Arthritis

From the total of 338 patients (data from 2 patients were missing; 1 EOP and 1 LOP), PsA occurred almost equally in both groups (25.3% of EOP patients and 23% of LOP patients). To better describe the relationship between PsA and the different subtypes of psoriasis (EOP vs LOP), a logistic regression model was built with PsA as the dependant variable (outcome), while the type of psoriasis (EOP or LOP), gender, disease severity, the number of involved nails and the nail changes were the independent variables. It was demonstrated that only the number of nails was significantly associated with PsA. In particular, the increase of the number of nails by 1 was associated with a 13% higher likelihood of developing PsA *(OR=1.13, P=0.009, 95% CI 1.03-1.24)*.

3.4.4.2 Type II Diabetes Mellitus

A total of 58 patients, out of 338 (data from 2 patients were missing; 1EOP and 1LOP), were affected with T2DM and none with type 1 Diabetes (T1DM). Overall, LOP patients were more likely to suffer from T2DM compared to EOP patients; 25 EOP patients (9%) and 23 (37.7%) LOP patients, x^2, *P<0.001*. At this point, it is noted that all LOP patients are aged >50 years, while EOP participants ranged from 18 to 90 years. It is known that the risk of developing T2DM increases with age and peaks after the age of 50 years. To detect whether the previous relationship was mainly affected by the age of the participants and hence the current results were a false positive, data were segregated by age. By including in the analysis, only patients aged 50 years and above (N=200; 139 EOP and 61 LOP), it was observed that the relationship between T2DM and LOP still remained statistically significant [22 EOP patients (15.8%) vs 23 LOP patients (37.7%); x^2, *P=0.001)*. To examine whether LOP is a significant factor for developing T2DM in patients over the age of 50 years, a logistic regression was computed. The dependent variable (outcome) was the binary marker T2DM (Yes or No), while the main independent variable comprised of the type of psoriasis (EOP or LOP). To adjust for confounders, the history of IHD, in combination with the risk variables for metabolic syndrome (HTN, dyslipidemia, central obesity), disease duration and the severity of psoriasis, were included in the model. It was observed that disease duration, IHD, high BP and dyslipidemia were not significantly related to T2DM. On the other hand, the type of psoriasis and BMI were significantly related to T2DM. More specifically, it was observed that, by an increase of 1Unit in BMI, there was a one-fold increase in the likelihood of developing T2DM *(logistic regression; OR_{BMI}= 1.076, P=0.011, 95% CI 1.017-1.139)*. In addition, there was approximately a three-fold higher chance in developing T2DM for LOP patients *(logistic regression; OR_{LOP}= 3.426, P=0.049, 95% CI 1.004-11.691);* (**Table 3.7**). Finally, there was a trend toward significance for a higher incidence of T2DM in patients with severe disease *(logistic regression; $OR_{Severe\ disease}$= 2.542, P=0.083, 95% CI 0.885-7.302).*

To further examine whether the previous effect between LOP and T2DM was also present when compared with the general population, a logistic regression model was employed with the same covariates as before, but the independent variable now included three categories; EOP, LOP and controls. By including data from the general population, it was expected that the previous wide 95%CI to narrow down, as the current data would now reflect the potential likelihood against the general population. A total of 357 patients, aged 50 years or more, were included in this analysis (157 controls, 139 EOP and 61 LOP) The current findings demonstrated that, compared to controls, LOP patients had approximately a three-fold greater likelihood of developing T2DM and this relationship was statistically significant *(logistic regression; OR_{LOP}= 2.56, P=0.050, 95% CI 1.001-6.54)*. EOP patients were found to have approximately a two-fold higher chance of T2DM, but this difference was non-statistically significant compared to controls *(logistic regression; OR_{EOP}= 1.561, P=0.67, 95% CI 0.340-7.170)*. In addition, BMI and HTN were found to be significantly linked to T2DM *(logistic regression; OR_{BMI}= 1.066, P=0.002, 95% CI 1.023-1.111 and OR_{HTN}= 2.549, P=0.008, 95% CI 1.271-5.112).*

3.4.4.3 Autoimmune Diseases

Crohn's disease and AIT were assessed in relation to EOP and LOP. Interestingly, although Crohn's disease was solely seen in EOP patients (1.4% of EOP subjects), this was not statistically significant *(independent proportions; P=1.0)*. On the other hand, AIT was more frequent in LOP patients [11 EOP(4%) vs 10 LOP (16.7%) patients*; Fisher's Exact Test; P=0.002]*. Again, as AIT is more frequent in middle aged patients, the psoriasis sample was stratified for age. Again, it was noted that the previous association remained significant [5 EOP (3.6%) vs 10 LOP (16.7%) patients; *Fisher's Exact Test, P=0.003].*

As with the T2DM, a logistic regression model was computed to examine the previous association. Gender, dyslipidemia, disease duration and severity were encountered as confounding variables. The type of psoriasis was treated as independent factor, while the AIT was the outcome. It was observed that patients with LOP had a significantly higher chance in developing AIT compared to EOP patients; *logistic regression, OR=5.05, P=0.005, 95% CI 1.62-15.7*. Gender, disease duration and severity, as well as dyslipidemia didn't not have any significant effect in the model.

The risk for AIT was assessed between the psoriasis groups and the general population. It was demonstrated that, compared to controls, EOP patients were approximately 97% less likely to develop AIT *(logistic regression; OR=0.025, P=0.020, 95% CI 0.001-0.55)*. On the other hand, LOP had only 5% lower likelihood for AIT, compared to controls, but this difference was non-significant *(logistic regression; OR=0.945, P=0.930, 95% CI 0.264-3.380)*. It was noted that the P value in the previous result was very close to 1, which probably implies that the risk for AIT in LOP subjects was similar to that in the controls group.

3.4.4.4 Other comorbidities

Patients were also asked whether they were ever diagnosed for atopic dermatitis, asthma, allergic rhinitis or allergic conjunctivitis. There was no significant relationship between the two study groups and atopy; x^2*, P=0.2* (**Table 3.7**).

In addition, the relationship between history of malignancy and psoriasis was assessed. Again, there was no significant difference between the two subtypes of psoriasis; *Fisher's exact Test, P=1.0* (**Table 3.7**).

Moreover, potential links of cardiovascular disease and the type of psoriasis were explored in patients ≥50 years of age; there was no significant association between age of onset of psoriasis and IHD (*logistic regression; OR=2.47, P=0.61, 95% CI 0.78-78.76*); **Table 3.7**.

Finally, obesity was evaluated based on the BMI of each patient, whilst waist circumference was treated as a covariate. Data of patients over the age of 50 years were examined; there was no identified link between high BMI and the type of psoriasis *(logistic regression; OR=1.54, P=0.66, 95% CI 0.23-10.36)*; (**Table 3.7**).

Clinical characteristics	EOP (N=278) (%)	LOP (N=62) (%)	OR	Adj. OR	P value	95% CI
Familial predisposition						
No family history of psoriasis[1]	105 (38%)	38 (64.4%)	-	-	-	-
Affected parent(s) with EOP	19 (26%)[2]	1 (3.1%)[2]	0.10	0.093	0.025*	0.01-0.74
Affected parents with LOP	3 (4.1%)[2]	4 (12.5%)[2]	3.5	3.52	0.16	0.59-20.7
1st degree affected relatives	88 (31.9%)	14 (23.7%)	0.44	0.41	0.011*	0.22-0.86
2nd degree affected relatives	42 (15.2%)	5 (8.5%)	0.33	0.30	0.018*	0.12-0.89
Both 1st and 2nd degree affected relatives	41 (14.9%)	2 (3.4%)	0.14	0.12	0.005*	0.03-0.58
Total of cases	276	59				
Missing cases	2	3				
Disease severity[3]						
Non-severe[1]	187 (69%)	48 (84.2%)	-	-	-	-
Severe	84 (31%)	9 (15.8%)	0.42	0.51	0.121	0.22-1.2
Total of cases	271	57				
Missing cases	6	5				
Nail Involvement[2]						
No nail involvement[1]	28 (36.8%)	8 (25%)	-	-	-	-
Changes of the nail plate	15 (19.7%)	8 (25%)	1.87	1.9	0.3	0.58-5.96
Changes of the nail bed	8 (10.5%)	10 (31.2%)	4.38	4.33	0.02*	1.27-14.8
Both	24 (31.5%)	5 (15.6%)	0.73	0.72	0.61	0.2-2.56
Total of cases	76	32				
Missing	0	0				

Table 3.6 Clinical comparison of early onset (EOP) versus late onset psoriasis (LOP) patients (N=340 patients).

The table summarises the main results on identified clinical and demographic differences between EOP and LOP, from the total of 340 patients. The column which presents the odds ratio (OR) refers to the first level of regression analysis, which uses binary logistic regression with no confounders or age stratification and illustrates the association between one independent variable and the outcome variable, which in this case is the type of psoriasis. The column with the adjusted (Adj.) OR represents the second level of regression analysis, where confounders are included in the model. [1]This is the reference category and hence no OR is computed, [2]the analysis is restricted to the prospective data only, as there was no similar information in the retrospective data. [3]Disease severity was computed as a binary variable (non-severe-0 and severe-1), which enabled as to the potential direct effect of severe skin disease on the type of psoriasis. Asterisk (*) represents statistically significant ORs.

CI=confidence interval

Psoriasis associated comorbidities	EOP (N=278) %	LOP (N=62) %	OR_{LOP}	Adj. OR_{LOP}	P value	95% CI
T2DM[1]	22 (15.8%)	23 (37.7%)	3.22	3.43	0.049*	1.004-11.69
IHD[1]	15 (10.8%)	5 (8.1%)	5.35	3.41	0.31	0.31-37.2
HTN[1]	68 (48.9%)	33 (54.1%)	1.23	1.16	0.65	0.62-2.18
Dyslipidemia[1]	36 (25.9%)	19 (31.1%)	1.33	1.13	0.75	0.38-1.49
Obesity (BMI)[1]	59 (44.7%)	29 (49.2%)	1.2	1.54	0.66	0.23-10.36
PsA	70 (25.3%)	14 (23%)	0.88	1.13	0.74	0.54-2.35
Crohn's disease	4 (1.4%)	0 (0%)	-	-	-	-
AIT[1]	5 (3.6%)	10 (16.7%)	5.36	5.05	0.005*	1.62-15.7
Atopy	69 (24.9%)	11 (18.3%)	0.68	0.65	0.28	0.31-1.42
Malignancy	10 (3.6%)	2 (3.2%)	0.91	2.69	0.31	0.4-17.98
Total of cases	277	61				
Missing cases	1	1				

Table 3.7 Psoriasis associated comorbidities and their relation to early onset (EOP) and late onset psoriasis (LOP); N=340.

The table summarises the main results from the total of 340 patients. The column which presents the odds ratio (OR) refers to the first level of regression analysis, which uses binary logistic regression with no confounders or age stratification and illustrates the association between EOP and LOP and the outcome variable, which in this case is the different comorbidities. The column with the adjusted (Adj.) OR represents the second level of regression analysis, where confounders are included in the model. The reference category is EOP and hence no odds ratio (OR) is computed. [1]Stratification by age has been applied and patients of 50 years and above are included in the analysis (N_{EOP}=139, N_{LOP}=61). Asterisk (*) represents statistically significant ORs.

T2DM= Type 2 Diabetes Mellitus, IHD= Ischaemic heart disease, HTN= hypertension, BMI= body mass index, PsA= Psoriatic Arthritis, AIT= autoimmune thyroiditis, CI= confidence interval

3.4.5 Psychological impact of the disease in early and late onset psoriasis

Psychological impairment in QoL and mood has long been linked to psoriasis. To examine for potential differences in QoL and mood between EOP and LOP, the psychometric scores of the self-reported DLQI, HADS, PSWQ and BDI were analysed.

Initially, the analysis included correlations between generic and more specific psychometric measures of psychological distress (DLQI and HADS-A, DLQI and PSQW, as well as HADS-A and PSWQ). By doing so, it was investigated whether the generic DLQI can detect mood disorders and whether HADS-A and PSWQ are both sensitive for detecting anxiety. It was observed that DLQI poorly detected worry *(Spearman's ρ=0.354 between PSWQ and DLQI)*, while there was a moderate correlation between DLQI and HADS-A *(Spearman's ρ=0.509 between HADS-A and DLQI).* On the other hand, HADS-A and PSWQ *(Spearman's ρ=0.741)*, as well as HADS-D and

BDI-II *(Spearman's ρ=0.822)* were strongly correlated and hence, were able to sufficiently detect mood disorders. All correlations were statistically significant at the level of 0.001.

3.4.5.1 Impact of skin disease on quality of life

As shown in **Table 3.8**, there was no significant difference in the impact of psoriasis on daily life between the two subtypes of psoriasis (N=270; 220 EOP and 50 LOP, mean DLQI in EOP= 8.3 ± 7.5 and mean DLQI in LOP= 8.1 ± 7). Data from 70 patients were missing, all from the retrospective arm. Since the LOP groups consisted of patients over the age of 50, an adjustment for age was applied in the data and again there were no differences in the mean DLQI scores between EOP and LOP (mean DLQI for EOP 7.4±7.1 vs 7.6±7 for LOP patients*; t-test, t=0.22, P=0.83*). Looking at differences in the DLQI score between genders, a trend towards significance was observed in scoring higher in the DLQI for the female subjects, compared to male subjects (mean DLQI of male subjects of 7.3±7.4 vs 8.9±7.3 for female subjects; *t-test; t=1.78, P=0.076)*. When gender differences were individually assessed within the study groups, it was observed that female subjects had scored higher but this was not statistically significant (mean DLQI of EOP male subjects of 7.5±7.3 and mean DLQI of EOP female subjects of 9±7.7; *t-test; t=1.51, P= 0.13,* whilst mean DLQI of LOP male subjects of 6.7±8.1 and mean DLQI of LOP female subjects of 8.7±5.6; *t-test, t=0.99, P=0.33*). All mean DLQI scores shown in **Table 3.8** indicated a moderate impact on patients' life.

3.4.5.2 Mood disorders in early and late onset psoriasis patients

3.4.5.2.1 Anxiety

As shown in **Table 3.8**, LOP patients (N=31) from the prospective cohort had a higher mean HADS-A, representing clinically significant levels of anxiety. On the other hand, the EOP group (N=75) was not an anxious group *(mean HADS-A for LOP=8±5, mean HADS-A for EOP=6±5; t-test, t=1.85, P=0.068)*. In line with the previous findings, the PSWQ, which is a more sensitive and specific test for anxiety disorders, demonstrated that the LOP group was a clinically more anxious group vs the EOP group *(mean PSWQ for EOP=43±14 vs mean PSWQ for LOP=46±15; t=test, t=1.008, P=0.32)*. However, the previous mean difference was not significant between the two subtypes of psoriasis.

An adjustment for age ≥50 years was performed, to examine whether the previous findings remained unaffected (N=62; 32 EOP and 30 LOP). Interestingly, the mean difference in HADS-A increased from 2 to 3, reflecting a statistically significant difference between the groups (mean HADS-A for EOP= 5 ± 5 and mean HADS-A for LOP=8 ± 5*; t-test; t=2.83, P=0.006*). Similarly, the mean score of PSWQ was higher in LOP, compared to the EOP group (mean PSWQ for EOP=40 ± 13 and mean PSWQ for LOP=46 ± 15*; t-test, t=1.82, P=0.074*).

By looking at gender differences, it was observed that females had significantly higher anxiety levels compared to males (mean HADS-A for male=6 ± 5 and mean HADS-A for female=8 ± 5*; t-test, t=2.21, P=0.029,* while mean PSWQ for male=41 ± 13 vs mean PSWQ for female=48 ± 16; *t-test, t=2.56, P=0.012).*

3.4.5.2.2 Depression

Psoriasis has been previously linked to depression. To detect, whether depression is specifically associated to either or both subtypes of psoriasis, the prospective data of HADS-D were analysed. Most notable, the means HADS-D in both groups was less than 8 and hence, didn't show any statistically significant levels of depression (**Table 3.8**). Only 42 EOP patients and 14 LOP patients scored ≥ 8 in the HADS-D and they were, hence, asked to complete a BDI-II. As seen in **Table 3.8**, the mean BDI-II scores again didn't show a statistically significant mean difference between groups.

An age adjustment was applied in the data and only patients ≥50y old were included in the analysis (N=62; 32 EOP and 30 LOP). Interestingly, the difference in the means of HADS-D increased and became statistically significant (mean HADS-D for EOP=2 ± 3 and mean HADS-D for LOP= 5 ± 4*; t-test, t=3.17, P=0.003*). This difference, although notable, didn't relate to increased depression levels in any group, as both mean scores were still below 8. Similar results were obtained for BDI-II (mean BDI-II for EOP 9 ±9, vs 14±12 in LOP*; t-test, t=1.4, P=0.19*).

By adjusting for gender, no significant differences were detected in neither HADS-D nor BDI-II scores (mean HADS-D for males of 4±4, vs 5±4 in females; *t-test, t=0.86, P=0.39* and mean BDI-II in males of 12±10 vs 14±12 in females; *t-test, t=0.64, P=0.53*) .

Finally, clinical information on history of diagnosed depression, from both psoriasis cohorts (prospective and retrospective), revealed that 38 EOP (13.7%) and 9 LOP (15,5%) patients had been diagnosed with depression (x^2*; P=0.720*).

Psychological Assessment Tools	EOP Mean ± SD	LOP Mean ± SD	t statistics	P value
QoL[1]				
DLQI	8 ± 8	8 ± 7	0.8	0.92
Mood disorders[2]				
Anxiety : HADS-A	6 ± 5	8 ± 5	1.85	0.068
PSWQ	43 ± 14	46 ± 15	1.008	0.32
Depression: HADS-D	4 ± 4	5 ± 4	1.13	0.26
BDI-II	13 ± 11	14 ± 12	0.43	0.67

Table 3.8 Mean scores of the psychometric questionnaires completed by the psoriasis participants.

[1]N=270 (data from both psoriasis cohorts), [2]N=106 (data from the prospective cohort)

EOP= early onset psoriasis, LOP= late onset psoriasis, SD= standard deviation, QoL=quality of life, DLQI=Dermatology life quality index, HADS-A=anxiety subscale of the hospital anxiety and depression questionnaire, HADS-D=depression subscale of the hospital anxiety and depression questionnaire, PSWQ=Penn state worry questionnaire.

3.5 Discussion

Henseler and Christopher's divided plaque psoriasis in two distinct sub-phenotypes, according to the age of the disease onset; EOP and LOP (Henseler and Christophers, 1985). Subsequently, various studies have demonstrated clinical and genetic differences between EOP and LOP. In this study, an extensive clinical comparison is reported, of 278 EOP patients and 64 LOP patients.

The current data confirm the concept of two distinct clinical phenotypes of psoriasis, based on the age of onset. It was therefore confirmed that EOP represents a familial form of psoriasis; 62% of EOP patients had at least one family member affected with psoriasis vs 35.6% of LOP subjects. This finding is in line with previous reports (Gudjonsson et al., 2006a; Mallbris et al., 2005; Henseler and Christophers, 1985; Ferrandiz et al., 2002). An interesting observation was that patients with a positive family history in the 1st and/or 2nd degree relative(s) were more likely to develop EOP compared to patients with no family history of psoriasis. As EOP showed a strong inheritance pattern in the prospective cohort, it was further investigated the link between the age of onset of the study subjects and the age of onset of their affected parents. Previous research has shown that patients with parental psoriasis are more likely to develop the disease early in life, especially when both parents are affected (Holgate, 1975). The current study showed that patients who had at least one affected parent with EOP, had 91% less likelihood in developing LOP compared to subjects with no affected parents and this was statistically significant **(Table 3.6)**. In addition, LOP parents, were approximately 4 times more likely to have LOP offspring, although the

previous finding was not statistically significant (**Table 3.6**). This finding may in part be explained by the lack of power to show significance given the small total number for LOP psoriasis patients. It would have been ideal, if there were similar data on age of onset of parents in the retrospective cohort and it was thus possible to include all 340 in this analysis. A larger cohort would be required to confirm these interesting, preliminary results. The effect of the age of onset of affected parents in their offspring is observed for the first time and is in line with previous observations that age of onset of psoriasis has a strong genetic background.

Chronic plaque psoriasis was the most common clinical form of psoriasis between both study groups; 86.7% of EOP patients and 88.1% of LOP patients; **Table 3.4**. There was no significant relationship between other clinical phenotypes with either EOP or LOP apart from a trend towards significance in suffering from scalp psoriasis in LOP subjects and as expected, from guttate psoriasis in EOP subjects; **Table 3.4**. In addition, although not statistically significant, small thick plaque, guttate, pustular and erythrodermic psoriasis were exclusively seen in EOP patients. This is partially in line with previous reports that showed significant associations of EOP with guttate and eruptive psoriasis (Gudjonsson et al., 2006a; Ferrandiz et al., 2002; Mallbris et al., 2005). However, the thin-plaque phenotype was more frequent in LOP patients (**Figure 3.4**). The aforementioned findings have not been reported in previous studies and hence further research is needed to validate these results. It would be interesting to know whether LOP is more linked to a thin-plaque phenotype which could thus potentially affect response to various therapeutic agents. Previous research on thin-plaque psoriasis has demonstrated that this phenotype is linked with guttate psoriasis, atopy and increased prevalence for malignancy (Christensen et al., 2006). The authors employed a x^2 analysis, usually applied in a 2x2 table, to identify these links, although the outcome variable (phenotype of psoriasis) consisted of 3 categories (thin-plaque, intermediate and thick-plaque). Such associations were established in this study, whilst the statistical analysis was based in a logistic regression model, which allows for multiple category variables (≥2 categories).

This study also confirmed observations from previous studies that LOP is a less severe disease compared to EOP which more often requires systemic treatment (especially biologic agents); (Kwon et al., 2012; Ferrandiz et al., 2002; Gudjonsson et al., 2002; Gudjonsson et al., 2006b; Di Lernia and Ficarelli, 2012). Instead of using the conventional PASI or BSA to assess disease severity, a different assessment of disease severity was employed. Both PASI and BSA are useful when assessing disease severity during a specific treatment, but they are not reliable for patients using different therapies. In particular, psoriasis patients on 2nd-3rd line treatment, with a PASI below 10, which indicates a mild to moderate disease, following response to systemic treatment, are not considered as having the same disease severity as those on topical treatment and mild psoriasis (PASI<10). A severity tool was therefore used, which, in addition to the extent of the skin disease, included current treatment, as well as emotional and self-reported disability. The severity tool was based on the one used by Gudjonsson et al, whilst it included an additional feature (the psychological impact of psoriasis, as measured by DLQI and PGPA); (Gudjonsson et al., 2002). Patients on systemic treatments were therefore categorised in two severity groups, the moderate to severe and severe disease group, based on their psychological distress levels, irrespective of PASI. Patients on topical treatment were grouped in mild to moderate, moderate to severe and severe group, based on their PASI and psychometric scores.

When the different anti-psoriatic treatments were compared, EOP patients were more frequently treated with continuous systemic treatment (oral systemic and/or biologic agents), irrespective of disease duration. On the other hand, LOP patients, were more commonly receiving phototherapy or topical treatment. These results concur with previous reports on the topic and reinforce the finding that LOP is a less severe disease (Ferrándiz et al., 2001; Ferrandiz et al., 2002; Kwon et al., 2012). It is known that thin-plaque psoriasis tends to respond and even clear-up with phototherapy (Zanolli, 2004). The increased prevalence of thin-plaque psoriasis (either small or large plaque) in LOP patients, could potentially explain the increased "preference" and effectiveness for phototherapy in LOP. The other side of the coin could be the older age of LOP patients and hence higher likelihood for comorbidities which makes dermatologists more conservative in prescribing systemic agents to such patients. However, after stratifying by age and including data from EOP and LOP patients over the age of 50 years, the aforementioned statistically significant relationships remained the same and hence age is not the main factor for LOP receiving phototherapy and EOP biologics in the study samples.

The current results also showed that EOP patients more frequently experienced exacerbation of their disease following URTIs compared to LOP patients. In line with previous research, stress was the most common triggering factor of flares in both groups, while anti-psoriatic treatment and sunny holidays were found to improve skin disease (Kotrulja et al., 2010).

Nail involvement was present in 88.9% of patients. Contrary to other reports which connect nail psoriasis to EOP, both groups had similar occurrence of nail changes (Ferrandiz et al., 2002; Henseler and Christophers, 1985). Interestingly, changes of solely the nail bed were more common in LOP patients. These changes included onycholysis and hyperkeratosis. In accordance to findings on *HLA-Cw*06:02* negative patients, the study subjects with nail bed changes had a fourfold increased probability in being LOP compared to those with no nail disease (Gudjonsson et al., 2006a; Gudjonsson et al., 2002); (**Table 3.6**). These findings are intriguing, as current literature suggests that LOP is linked with PsA, which is separately associated with nail matrix changes (McGonagle, 2009; Ferrandiz et al., 2002). No such a link was indentified in the study sample.

Previous studies have linked psoriasis to a number of comorbidities. This is the first study which extensively explored links between psoriasis comorbidities and the two subtypes of psoriasis. It was shown that PsA was present in 24.7% of the total of 340 patients (25.3% of EOP patients and 23% of LOP patients). It was confirmed that PsA is significantly linked to the number of affected finger nails. More specifically, for every additional affected nail there is a 13% higher likelihood in developing PsA. As previously mentioned, the current findings did not find any direct associations between PsA and LOP.

In line with recently reported data (Armesto et al., 2012; Mazlin et al., 2012), T2DM was more common in LOP patients (**Table 3.7**). The risk for developing T2DM increases with age, while it is closely linked to metabolic syndrome and severe psoriasis (Huerta et al., 2007; Peter et al., 2007; Rosenberg et al., 2007). By adjusting for age, gender, BMI, HTN, dyslipidemia, IHD, disease severity and duration, a striking association was observed. In particular, it was demonstrated that patients suffering from LOP were 3 times more likely to develop T2DM compared to EOP patients (15.8% of EOP vs 37.7% of LOP patients, suffering from T2DM). To validate the aforementioned findings and examine whether the previous association is present vs the general population, data

on T2DM were included, from a control group of non-psoriasis subjects. Again it was shown that LOP patients had approximately threefold higher chance in developing T2DM, compared to the non-psoriasis population. However, although EOP patients also demonstrated an increased likelihood in developing T2DM compared to the general population, this association was not statistically significant. The main disadvantage of the two previous studies on the topic to support the link between T2DM and LOP, is that the authors did not state if they stratified their data by age (Mazlin et al., 2012; Armesto et al., 2012). This is important, because if their study groups were not age-matched, their EOP group would by default have a lower risk for T2DM, as it includes younger patients who, in general, have a lower likelihood of developing T2DM. Moreover, the Spanish study results, presented in tables, are spurious, especially the table which presents the risk for T2DM; the statistical significance is presented in OR, CI and P values, which indicates a multivariate analysis with regression models, although the legend of the table suggests that the analysis was carried out with x^2. This thesis is the first to account for confounding variables and encounter the age-dependant prevalence of T2DM. It is therefore encountered that the current analysis strategy is fairly robust to identify links between T2DM and type of psoriasis. The wide 95% CI (1.004-11.69) is more likely to be associated with a small sample size of LOP patients (N=61) and therefore, a larger study is required to confirm the current findings. Moreover, although it was found here that BMI is closely linked to T2DM, this study was unable to identify any associations between an increased BMI and/or waist circumference and the type of psoriasis (**Table 3.7**). The previous contradicts recent observations from a Hungarian group of researchers, that identified significant links between central obesity and LOP (Herédi et al., 2013).

In addition to the previous, another novel finding was the significant association of AIT with LOP disease (**Table 3.7**). More specifically, AIT was found to be less prevalent in EOP patients compared to controls. This is intriguing as psoriasis is directly linked with a higher risk for autoimmune diseases, and this is further supported by recent research which shows that immune-mediated, inflammatory conditions are linked with a common cytokine based pathology (Wu et al., 2012; Makredes et al., 2009; Robinson et al., 2006). Based on the previous, one would expect that psoriasis is associated with AIT. Few studies have, however, explored the prevalence of AIT in patients with psoriasis. A recent study from Turkey was the first to report high levels of thyroxine in patient with psoriasis compared to controls, irrespective of co-existing thyroid disease in these patients (Gul et al., 2009). Another study from Italy, associated clinical and subclinical AIT with PsA only (Antonelli et al., 2006). The current results were merely based on patient recall of having been diagnosed with thyroid dysfunction and relevant therapeutic management. However, a large, 25,341 patients, retrospective study from California, USA, exploring links between psoriasis and 21 common autoimmune diseases, was unable to establish a significant relationship between Hashimoto, Grave's disease and psoriasis (OR for Hashimoto: 1.2, 95% CI 0.9-1.5 and OR for Graves: 1.1, 95% CI 0.9-1.3); (Wu et al., 2012). The authors didn't account for age of onset of psoriasis in their subjects, nevertheless, theirs results partially confirm the ones presented in this thesis. Further research is needed to confirm the current results and speculate whether age of onset of psoriasis has a "protective" role against AIT.

Previous studies have underlined the strong association between smoking and alcohol abuse and psoriasis (Farkas and Kemény, 2010; Huerta et al., 2007; Kirby et al., 2008; Monk and Neill, 1986;

Naldi et al., 1992). Smoking is related to a number of adverse health effect, while alcoholism is a systemic disease, which affects several psychological domains (Bajaj et al., 2008; Yanbaeva et al., 2007). In the current results, there were no such links observed among the number of cigarettes smoked per day, the units of alcohol per week and the development of either EOP or LOP. This is perhaps related to the fact that patients don't fully disclose the extend of smoking and alcohol abuse, when asked. The previous highlights the importance of using objective measures to accurately assess alcohol and smoking intake.

There are many studies associating psoriasis with stigma and discrimination, which lead to psychological impairment (Da Silva et al., 2006; Gupta and Gupta, 1995; Langley et al., 2005; Pearce et al., 2006; Richards et al., 2001; Devrimci-Ozguven et al., 2000). Few studies, however, have examined psychological differences between EOP and LOP (Gupta et al., 1996; Kotrulja et al., 2010; Remröd et al., 2013a). The results of this thesis, from the psoriasis cohorts, showed that LOP patients were a clinically anxious group of patients when compared to age matched EOP subjects and this was statistically significant; **Table 3.8**. These findings contradicted previous reports which showed that anxiety levels did not significantly differ between EOP and LOP (Kotrulja et al., 2010). In addition, a recent Swedish study demonstrated that clinical levels of anxiety are more frequently seen in EOP patients; a results which opposes the current observations (Remröd et al., 2013a). The authors classified their patients based on age of onset being less (EOP) or above (LOP) 20 years of age, whilst they included patients of 18-65 years of age. This study, based on the original study from Henseler and Christophers, uses the cut off age of onset of 40 years for EOP patients, while LOP includes those with age of onset ≥ 50 years of age and hence the results presented here may not be comparable to those of the recent Swedish study (Henseler and Christophers, 1985). Moreover, contrary to previous research (Kotrulja et al., 2010; Ferrandiz et al., 2002; Henseler and Christophers, 1985), both EOP and LOP patients had mild symptoms of depression, while their QoL was moderately impacted by their skin disease; **Table 3.8**. It should be noted here that the mean BDI-II score for LOP patients was 14 (cut-off is 13) and hence, although it indicates mild depression levels, is above the cut-off point, compared to EOP patients with a mean score of 9.

Comparison of PASI and DLQI showed that clinical severity was not a good indicator of detecting psychosocial impairment and this is in line with other reports (Kotrulja et al., 2010; Fortune et al., 1997). In addition, DLQI and HADS were moderately correlated with each other, while DLQI was purely linked to PSWQ. The previous indicate that DLQI, although a good measure of QoL, is unable to detect mood disorders. On the other hand, HADS-A and PSWQ and HADS-D and BDI-II were strongly correlated and had increased sensitivity in detecting mood disorders (anxiety and depression). The current findings show that in addition to DLQI, a short psychometric screening measure which detects mood is needed in clinical practice, especially when assessing psoriasis patients. These tools can detect psychological distress and lead to a timely psychological intervention.

Several methodological factors need to be considered when assessing the data of this study. First, clinical information, especially on comorbidities and family history, was merely based on recall data. In addition, disease duration was significantly different between EOP and LOP patients (27.9y±13.7 for the EOP patients vs 11.86y±7.92 for the LOP patients; *independent t-test,*

t=11.004, P<0.001) and although it was included in the analysis as a confounding factor, it cannot be completely ruled out that some of the observed effects were due to disease duration instead of age of onset. Moreover, although patients were recruited from a tertiary psoriasis clinic, as well as from the Greater Manchester Primary Care Trusts (PCTs), the results are mostly on white British patients and may not apply to the general psoriasis population. Furthermore, there were no data on the genetic profile of the study participants. Nevertheless, this study supports the concept of two different phenotypes of psoriasis, based on the age of onset of psoriasis and presents new information on distinct clinical, demographic and psychological differences between the two subtypes of psoriasis.

4. A histological investigation of early and late onset psoriasis

4.1 Introduction

The main histological features of PP skin include epidermal hyperkeratosis, parakeratosis, acanthosis, loss of the granular layer, tortuous papillary vessels and elongation of the rete ridges. Neutrophilic collections are present in the stratum corneum and within the epidermis whilst lymphocytes are seen in both the epidermis and dermis (Du Vivier, 2002). Interestingly, both the clinical and histological appearance of psoriasis, from different anatomic locations, can vary in the same patient.

In addition, PN from psoriasis patients demonstrates subtle differences from normal skin, with lymphocytes and monocytes accumulating in the upper dermis, whilst mild spongiosis is seen in the epidermis and a few foci of dilated blood vessels in the dermis (Van De Kerkhof, 2007; Schubert and Christophers, 1985; Krueger et al., 1981).

Histology also varies among the different clinical variants of psoriasis. For example, although also present in plaque psoriasis, guttate psoriasis often exhibits the phenomenon of “squirting papillae”, where neutrophils are intermittently released from the dilated papillary capillaries, migrate into the epidermis, at the areas of parakeratosis and form Munro microabscesses (Krengel et al., 1998). Since the areas of parakeratosis are formed in different time points in guttate psoriasis, a psoriasiform pattern with alternate areas of parakeratotic and orthokeratotic stratum corneum is often seen (Krengel et al., 1998). In addition, the thinning of the suprapapillary plate is minimal to moderate, while the absence of the granular layer is often patchy. Pustular psoriasis has a marked epidermal neutrophilic infiltrate, which forms spongiform networks in the granular layer - Kogoj pustules (Valdimarsson et al., 1986; Krengel et al., 1998). A diffuse lymphocytic infiltrate is often present in the dermis.

The accurate histological diagnosis of psoriasis can sometimes be obscure; a wide variety of skin conditions clinicopathologically mimic psoriasis and hence are grouped into psoriasiform dermatoses (Barr and Young, 1985). These include seborrhoeic dermatitis, pitiriasis rubra pilaris (PRP), superficial fungal infections (dermatophytoses), psoriasiform drug reactions, lichen simplex chronicus and mycosis fungoides. In standard textbooks, the presence of Munro and Kogoj’s pustules and the thinning of the suprapapillary plate are more specific for psoriasis, whilst the presence of epidermal spongiosis and dilated blood vessels, as well as irregular hyperplasia and absence of Monro and/or Kogoj's pustules are less specific. There are very few studies examining the specificity and sensitivity of individual histological characteristics or a combination of these characteristics for psoriasis (Trozak, 1994; Beek and Reede, 1977). For this reason, additional special stains and IHC tests are required to differentiate psoriasis from other forms of psoriasiform dermatitis. Such tests include the Periodic acid- Schiff stain, used to rule out superficial fungal infections.

The classification of psoriasis discussed in this thesis, recognises two different phenotypes of psoriasis; EOP and LOP. Whereas the clinical heterogeneity between EOP and LOP has been extensively discussed in the literature, the histological distinction and evaluation of these two subtypes of psoriasis is yet to be explored.

4.2 Objectives

The primary objective of this study was to explore potential histological differences between EOP and LOP. Specific aims include:

- To compare epidermal and dermal histomorphological features in both PP and PN skin, taken from EOP and LOP patients.
- To assess the epidermal and dermal inflammatory infiltrate of PP and PN skin from EOP and LOP patients.

4.3 Methods

A skin biopsy study was conducted in a hospital and community-based, Caucasian, age-matched population between the years of 2010 and 2012.

4.3.1 Study population and procedures

A total of 32 unrelated, white British patients with psoriasis were recruited. Patients were considered to have EOP, if their psoriasis was diagnosed before the age of 40 years and LOP, if the diagnosis of psoriasis was made after the age of 50 years. All patients were over the age of 50 years at the time of biopsy.

Patients were consented for participation in the study and then screened according the study specific criteria (**chapter 2, section 2.2.2.5**). They were considered eligible only if they met all inclusion and none of the exclusion criteria. Subsequently, a physical examination, skin assessment with PASI and previous medical history were obtained and recorded in the subject-specific site files. Patients then had four, 6 mm, skin biopsies, taken from sun-protected, upper buttock skin; two from PP and two from PN skin (at least 5 cm away from a plaque). Written and verbal information on wound care was provided to all volunteers and a letter to the GP was sent after the study visit was completed.

A pair of PP and PN biopsies was placed in 10% formalin, then processed to paraffin embedding and finally stained with H&E, at the Cellular Pathology Department at SRFT (**chapter 2, 2.2.4.1**). The rest of the biopsies were either placed in OCT embedded medium and then snap frozen in liquid nitrogen or were immediately snap-frozen, prior to their storage at -80^0 C.

4.3.2 Histological assessment and statistical analysis

The stained slides were randomised and assessed under a LEICA DM2000 light microscope (Leica Microsystems, UK). Both PP and PN slides were reviewed in a blinded fashion and findings were recorded in a study specific histological proforma, the psoriasis histological assessment score (**chapter 2, section 2.2.5**).

Both inflammatory and morphological features were examined in the epidermis and dermis. The inflammatory parameters were further divided into:

1. Cellular elements of inflammation in the epidermis; presence of ICMCs, epidermal neutrophils, epidermal lymphocytes and eosinophils.
2. Cellular elements of inflammation in the dermis; presence of dermal neutrophils, dermal lymphocytes, dermal eosinophils and dermal mast cells.

Similarly, the morphological features were classified in:

1. Structural features of the epidermis; total epidermal thickness, extent of hypogranulosis, epidermal parakeratosis, presence of acanthosis, elongation of rete ridges, thinning of the suprapapillary plate and epidermal spongiosis.
2. Structural features of the dermis; dermal papillary oedema and tortuous papillary blood vessels.

Six sections per biopsy were examined across the whole of the epidermis and dermis, per x200 and x400 magnification fields. A semi-quantitative grading of 0-3 (0=normal, 1=minimal, 2=moderate and 3=severe), was employed to score severity of inflammation and morphological changes. Individual scores were recorded in a spreadsheet and were added up to give the THS, which ranged from 0 to 39 (**chapter 2, section 2.2.5**). In addition, two separate scores were calculated; the TIS, which was obtained by summing all scores of the inflammatory parameters of the epidermis and dermis, and the TMS obtained by the sum of scores of the structural features of the epidermis and dermis.

The statistical analysis (**chapter 2, section 2.2.7**) comprised three *independent samples t-test models*; a *t-test* exploring potential, morphological differences in tissue architecture, another one examining potential differences in the inflammatory pattern and a last one comparing the THS of both PP and PN sections from EOP and LOP patients. For data that did not meet the normality criteria, a *Mann-Whitney* was used. *Chi-square* was used to compare categorical variables and *Fischer's exact test* was employed where x^2 was not applicable In addition, a *linear regression model* was computed to control for the following confounders; disease duration, gender, clinical phenotype and PASI. The default significance was set at $P<0.05$. The statistical analysis was carried out, using SPSS statistical software for Windows, release 20·0 (SPSS Inc., Armonk, NY: IBM Corp U.S.A.).

4.4 Results

4.4.1 Clinical and demographic characteristics

Data from 31 subjects were analysed. As there was inconsistency in terms of age of onset between information recall by subject 3 and his clinical notes, his data were excluded from the analysis. A total of 17 EOP patients (14 male, with a mean age of 58.8±7.9 and 3 female, with a mean age of 58.3±8.0) and 14 LOP subjects (7 male, with a mean age of 66.7±6.9 and 7 female, with a mean age of 64.3±8.2) were enrolled in the biopsy study. Clinical and demographic characteristics are detailed in **Table 4.1**. All 31 participants were Caucasian, aged 50 years or more, were on topical treatment for their psoriasis, which was washed out for 2 weeks prior to their participation in the study. In addition, mean PASI on the day of biopsies was below 10, which indicates mild disease (**Table 4.1**). Non-psoriasis medications included anti-hypertensive medications (angiotensin-converting-enzyme-ACE inhibitors, calcium channel blockers and diuretics), NSAIDs, mild analgesics, anti-depressants and anti-T2DM medications. The relevant past medical history of the study participants, is listed in **Table 4.1**. A LOP patient (subject 1) had recently stopped beta-blockers and switched over to ACE inhibitors.

Clinical and Demographic Data	Frequencies (%) and Means ± SD	
	EOP (N=17)	LOP (N=14)
Sex		
Male	14 (82%)	7 (50%)
Female	3 (18%)	7 (50%)
Age of subjects		
Male	58.8±7.9	66.7±6.9
Female	58.3±8.0	64.3±8.2
Age of onset of psoriasis		
Male	29.2±14.3	55.3±4.8
Female	28.0±23.6	57.4±9.7
Disease duration		
Male	31.1±16.9	11.4±6.8
Female	30.7±17.1	7.4±4.8
PASI		
Male	7.6±3.5	8.1±4.6
Female	6.9±3.2	8.9±3.7
Clinical Phenotype		
Large thick plaque psoriasis	10 (59%)	5 (36%)
Large thin plaque psoriasis	5 (29%)	7 (50%)
Small thick plaque psoriasis	2 (12%)	0
Small thin plaque psoriasis	0	2 (14%)
Comorbidities*		
T2DM	0	4 (29%)
IHD	1 (6%)	2 (14%)
HTN	8 (47%)	6 (43%)
AIT	1 (6%)	0
Dyslipidemia	4 (24%)	5 (36%)
Myasthenia Gravis	0	1(7%)
PsA	3 (18%)	3 (21%)
Depression	4 (24%)	4 (29%)
Asthma	1 (6%)	2 (14%)
Crohn's disease	1 (6%)	0

Table 4.1 Clinical and demographic characteristics of the biopsy cohort (N=31).

*Table 4.1 lists all clinical and demographic information of the biopsy study population. T2DM= type 2 Diabetes Mellitus, IHD=ischemic heart disease, HTN= hypertension, AIT=autoimmune thyroiditis, PsA=psoriatic arthritis. * The above frequencies of comorbidities do not sum up to 100% as a substantial number of subjects had one or more comorbidities at the time of biopsy.*

4.4.2 Histological comparison of uninvolved skin

The morphological and inflammatory characteristics of PN, H&E slides were examined across the whole 6 sections and a grading score was obtained for THS, TIS and TMS. The data did not pass the normality test (*Kolmogorov-Smirnov test*; P=0.004), hence the non-parametric *Mann-Whitney* was chosen over the *t-test*. The THS ranged from 0 to 6 and the median THS was 2±2 in both EOP and LOP sections *(Mann-Whitney, U=153, P=0.16)*.

4.4.2.1 Morphological characteristics of uninvolved skin

Uninvolved skin from both EOP and LOP patients showed minimal to no morphological changes. The vast majority of sections did not differ from normal skin. The TMS ranged from 0 to 4. Eight EOP and all 14 LOP samples showed minimal non-specific changes in architecture of the skin, such as mild and regular elongation of rete ridges, focal dilation of dermal vessels and mild epidermal spongiosis. The median TMS for the EOP group was 1±2 and 2±1 for the LOP group *(Mann-Whitney, U=153, P=0.16)*. The mean ETh was equal to 0.15 ± 0.05 mm in both EOP and LOP PN samples.

4.4.2.2 Inflammatory characteristics of uninvolved skin

The TIS ranged from 0 to 4, while the median TIS of both groups was equal to 1±1 *(Mann-Whitney, U=117.5, P=0.94).* Focal dermal infiltration of lymphocytes was the most frequently observed inflammatory change in PN sections of both groups. Interestingly, one EOP sample from a female participant exhibited a focal collection of ICMC, indicating a spongiosis-associated skin reaction (**Figure 4.1B**).

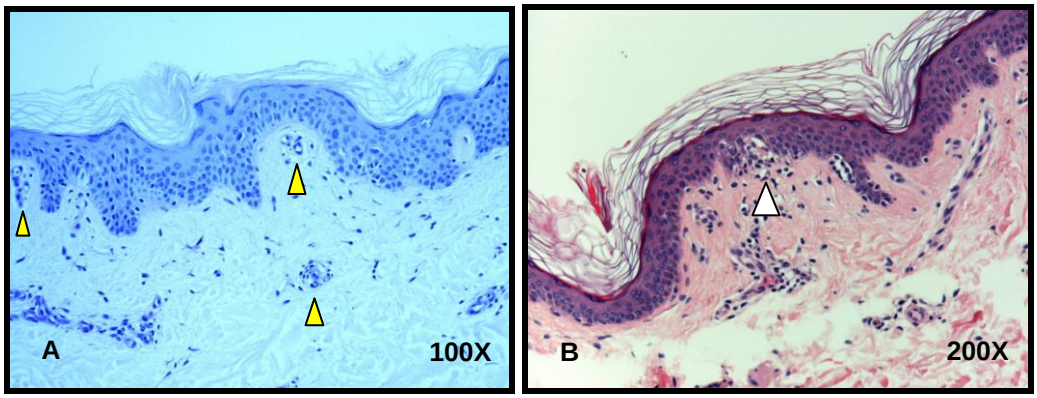

Figure 4.1 Histological features of uninvolved (PN) skin sections stained with periodic-acid-Schiff (PAS) and haematoxylin and eosin (H&E).

*Microphotographs of **(A)** PAS and **(B)** H&E from PN sections of psoriasis patients from this study and permission has been obtained. **(A)** This is a negative PAS microphotograph from late onset psoriasis (LOP). There are a couple of foci of dilated blood vessels with perivascular lymphocytic infiltration seen in the dermis (yellow arrowheads). In **(B)**, the white arrowhead points to a foci of non-lymphoid intraepidermal collections of mononuclear cells (ICMC). Surrounding dermal lymphocytes are gathered near the dermoepidermal junction. The microphotograph was obtained from an early onset psoriasis (EOP) section of skin.*

4.4.3 Histological comparison of involved skin

The histological components of PP, H&E sections of skin from EOP and LOP patients were assessed and compared. The THS, TMS, and TIS data were found to be normally distributed with the *Kolmogorov-Smirnov test* (P=0.456) and the *t-test* was employed to compare mean differences (1st level of analysis) and *linear regression* to detect associations (2nd level of analysis). The THS ranged from 14 to 37. A trend towards significance was observed when the mean THS were compared (*t-test, t=1.97, P=0.059*), with the mean THS in the LOP group (27±6) being higher than that of the EOP group (23±5); (**Table 4.2 and Figure 4.2**). In the 2nd level of analysis, the effect of the type of psoriasis (EOP or LOP) on the THS was examined as an independent variable, against several confounders (disease duration, clinical phenotype, gender and PASI), using a *linear regression test*. The purpose of this analysis was to ensure that the aforementioned impact on the mean THS was not driven by other factors. The THS was significantly related to LOP, irrespective of the other variables, in such a way that LOP was significantly linked to a higher THS (*regression; B coefficient=6, P=0.030, 95% CI 0.65-11.71*).

To examine which components of THS (the morphological or inflammatory component) were more related to the type of psoriasis, the THS score was broken down in TIS and TMS and separate analyses were carried out.

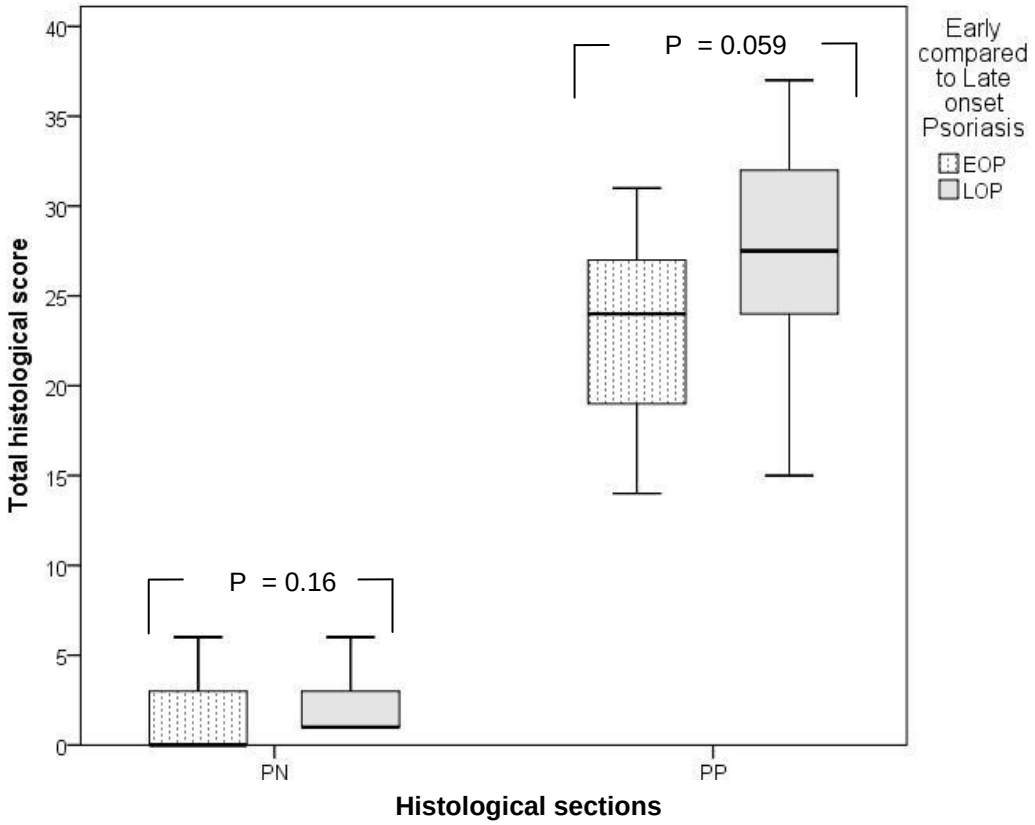

Figure 4.2 The total histological score (THS) of uninvolved (PN) and involved (PP) skin from early onset-EOP (N=17) and late onset psoriasis-LOP (N=14) patients.

This figure is a box and whisker diagram, which shows the median differences, as well as the minimum and maximum values of the THS in PN and PP skin from EOP and LOP patients. The significance level is also recorded.

4.4.3.1 Morphological features of involved skin

The TMS varied from a minimum of 10 to a maximum of 23, with the TMS of the EOP ranging from 10 to 21 and 11 to 23 for the LOP respectively. The mean TMS between the groups did not differ at a significance level of <0.05 (**Table 4.2**). The thick plaque psoriasis phenotype showed more severe histomorphological features (mean TMS 19±3) compared to thin plaque psoriasis (mean TMS 12±4), whilst the mean TMS for small and large plaque psoriasis did not show wide differences (mean TMS 14±6 and 15±5). The majority of samples demonstrated moderate to severe changes in the epidermal and dermal structure, with acanthosis being the most prominent structural change (97% of samples), whilst epidermal spongiosis was the least common (81% of samples). A linear regression did not reveal any correlation between TMS and the type of psoriasis or any of the confounding factors and hence confirmed the *t-test* results *(regression; B coefficient=3, P=0.097, 95% CI -0.59 - 6.7)*.

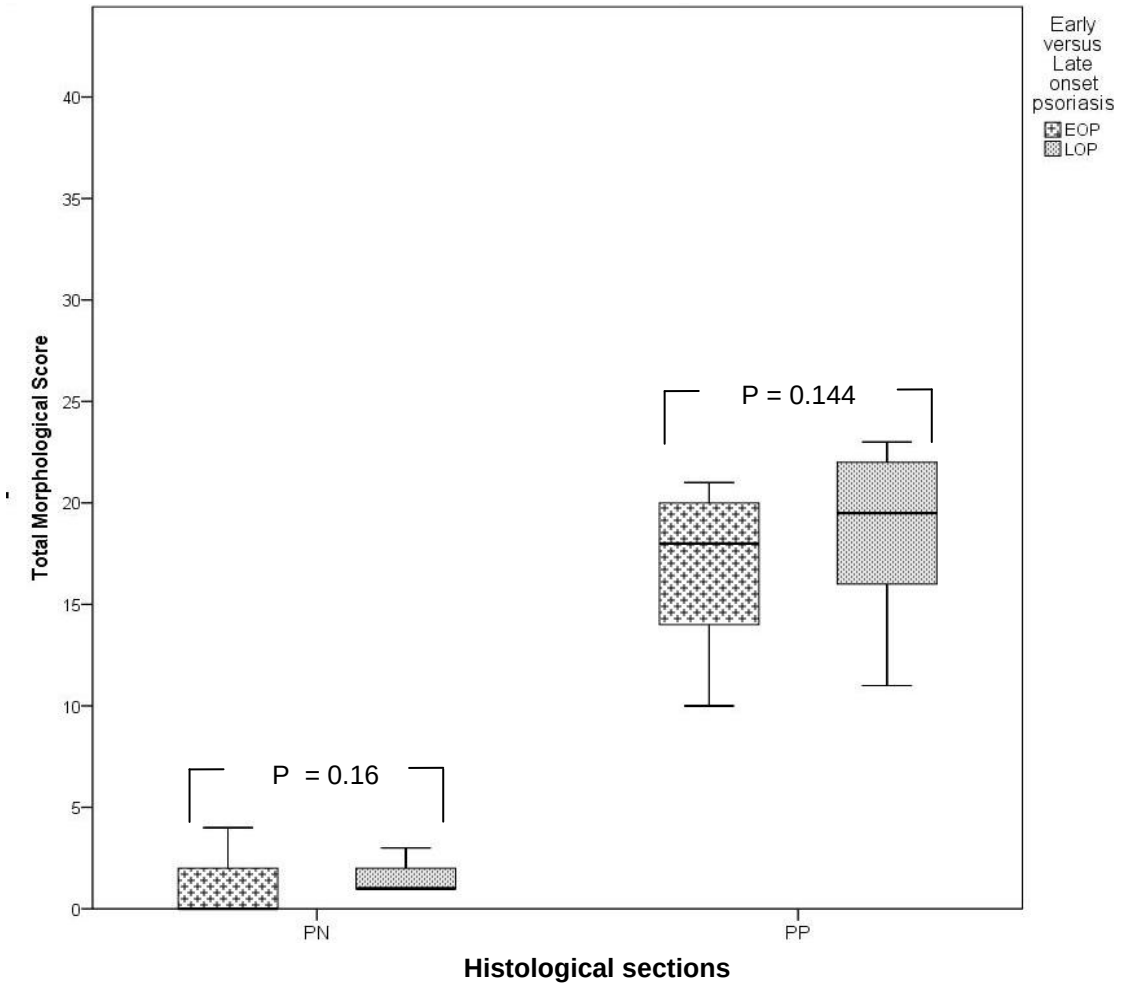

Figure 4.3 The total morphological score (TMS) of uninvolved (PN) and involved (PP) skin from early onset-EOP (N=17) and late onset psoriasis-LOP (N=14) patients.

This figure is a box and whisker graph, demonstrating the median differences, as well as the minimum and maximum values of the TMS in PN and PP skin from EOP and LOP patients. The significance level is also recorded.

4.4.3.2 Inflammatory features of involved skin

The TIS varied from 2 to 14; for the EOP group the TIS ranged from 2 to 11 and 4 to 14 for the LOP group respectively. A statistically significant difference was noted in the mean TIS between the two subtypes of psoriasis, with the LOP tissue sections showing more severe inflammation compared to the EOP samples (*t-test, t=2.32, P=0.028*); (**Table 4.2 and Figure 4.4**). Further microscopic inspection of the tissue sections, revealed that 50% of the LOP sections showed severe lymphocytic infiltration, while the rest had a moderate lymphocytic infiltrate across the epidermis. In addition, 43% of LOP sections also demonstrated severe lymphocytic infiltration of the dermis, whilst again the rest were limited to a moderate infiltration across the dermis. On the other hand, only 6% of the EOP sections exhibited severe dermal infiltrate of lymphocytes, with 70% of samples showing moderate, 18% minimal and 6% no infiltration across the dermis. The epidermal infiltrate ranged from patchy foci (moderate) of lymphocytes in 23%, to a few foci

(minimal), across the epidermis, in 77% of the EOP samples. The intensity of the neutrophilic infiltrate did not differ between the two subtypes of psoriasis. In addition, the presence of ICMC collections in the epidermis was seen in equal proportions between the compared groups. Moreover, there was no significant difference in the severity and presence of inflammatory elements among different clinical phenotypes. Finally, the presence of eosinophils in the dermis was noted in 29% of LOP and 41% of EOP samples *(Fischer's exact test, P=0.707),* whilst the extent of the eosinophilic infiltrate varied from mild to moderate.

A *linear regression* was modeled and showed that LOP was significantly linked to a high TIS when controlling for disease duration, gender, PASI and clinical phenotype *(regression, B coefficient=3, P=0.011, 95% CI 0.795 - 5.466)*.

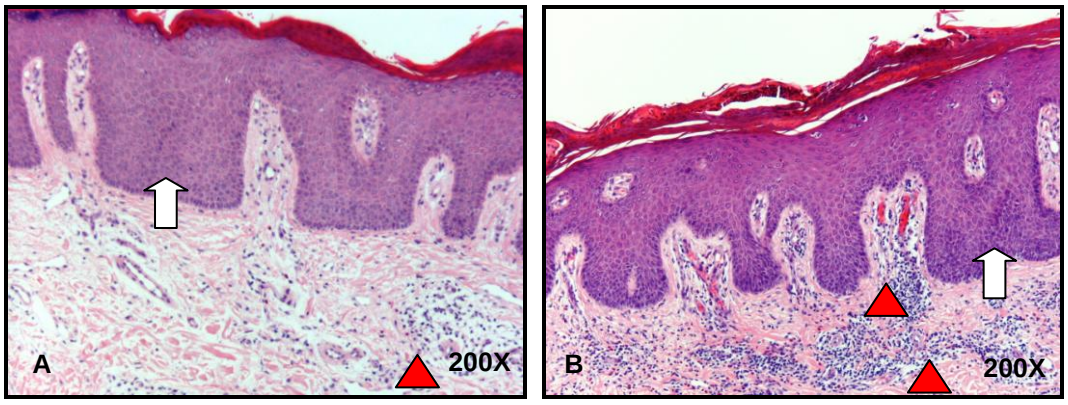

Figure 4.4 A comparison of histological features from early onset-EOP and late onset psoriasis-LOP skin sections.

*This figure shows the histological changes seen in psoriasis plaques from EOP (**A**) and LOP (**B**) patients. When comparing the photomicrographs, the histomorphological elements (white arrows) of the epidermis and dermis look alike, whereas the inflammatory infiltrate (red arrowheads), which is primarily lymphocytic, is more prominent in the LOP section.*

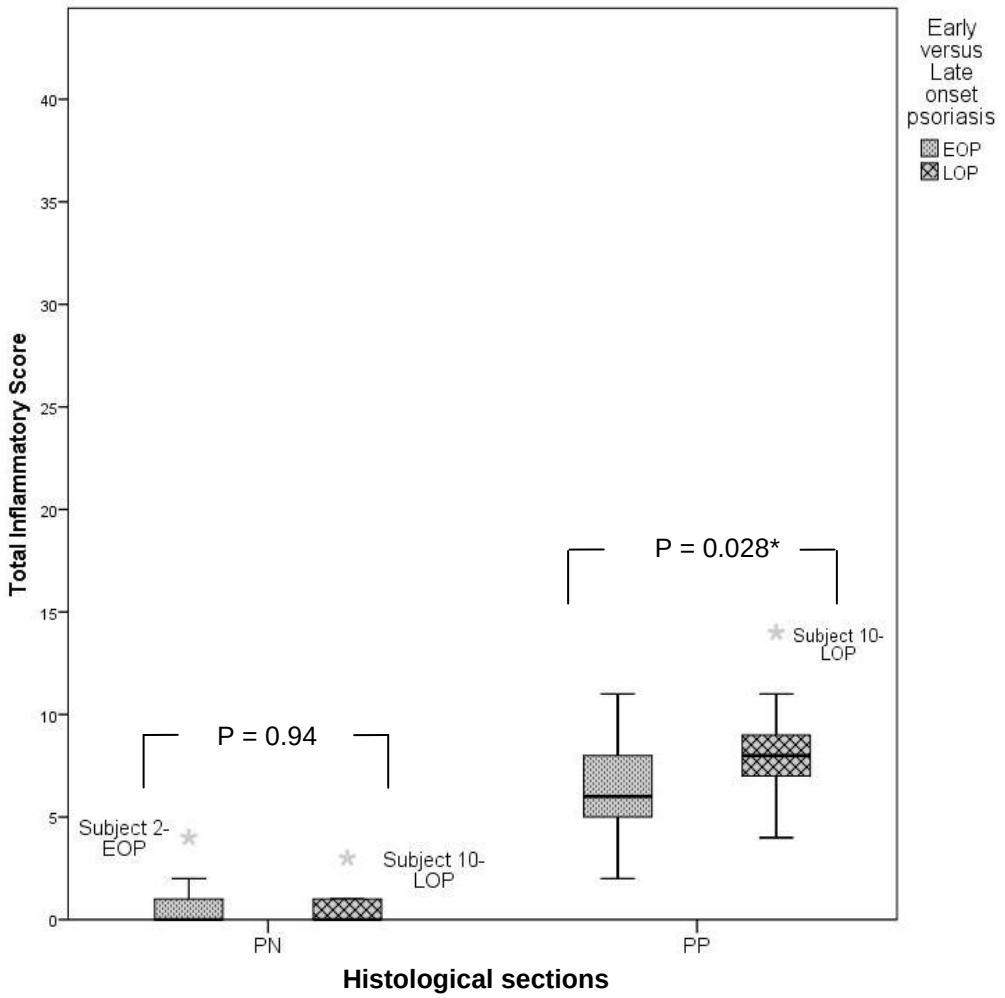

Figure 4.5 The total inflammatory score (TIS) in uninvolved (PN) and involved (PP) skin from early onset-EOP (N=17) and late onset psoriasis-LOP (N=14) patients.

This figure is a box and whisker graph, which presents the median differences, as well as the minimum and maximum values of the TIS in PN and PP skin from EOP and LOP patients. The significance level is also recorded. The black asterisk (*) represents a significance level < 0.05, while outliers are identified with a grey asterisk (*). In this t-test analysis, subject 10 is an outlier for the LOP group and demonstrates the highest TIS in both PN (TIS=3) and PP skin (TIS=14). Subject 2 is an outlier for the EOP group, only for the TIS, in PN sections (TIS=4). The EOP outlier was female, while the LOP outlier was male. The analysis was re-run without the outliers and the results remained the same.

Histological Scores PP samples	Compared Groups EOP (Mean ± SD)	LOP (Mean ± SD)	Comparison of Histological Scores Independent Samples t-test t-statistics	P value
THS	23±5	27±6	1.965	0.059
TMS	17±3	19±4	1.500	0.144
TIS	6±2	8±2	2.318	0.028*
Total Eth	0.35mm	0.43mm	1.124	0.276

Table 4.2 Comparison of the mean histological scores of involved (PP) skin samples from early onset-EOP (N=17) and late onset psoriasis-LOP patients (N=14).

This table demonstrates the mean differences and statistical significance of the total histological score (THS), the total morphological score (TMS) and total inflammatory score (TIS) from psoriasis plaques (PP) sections of EOP and LOP patients. In addition, the mean total epidermal thickness (Total Eth) is displayed at the end of the table.

** Statistically significant, SD=standard deviation, mm=millimetres*

4.5 Discussion

Psoriasis is a clinically and genetically heterogeneous skin disease for which, in daily practice, the diagnosis is based on clinical examination and medical history. However, biopsy and histological assessment is still required, especially where clinical diagnosis is difficult (Gutierrez et al., 2011).

Various studies exist which explore the clinical and genetic differences between EOP and LOP and have shown that compared to LOP, EOP is a clinically more severe form of psoriasis, linked to the *HLA-Cw*06:02* allele (Henseler and Christophers, 1985; Ferrandiz et al., 2002). Other studies have also examined differences in the immune response of certain inflammatory cells between these two subtypes of psoriasis and have found that in contrast to LOP, the epidermal LC migration is impaired in EOP PN samples as compared to LOP (Shaw et al., 2010).

Despite these data, there is no evidence on whether EOP and LOP are histologically different. This is the first study to examine histological differences between EOP and LOP, using skin sections from PP and PN. For the purpose of the study, 62 skin samples (31 PN and 31 PP) were stained with H&E and analysed under the microscope.

Microscopically, psoriasis is characterised by a psoriasiform reaction pattern and resembles a variety of other unrelated skin conditions (psoriasiform dermatoses); (Krengel et al., 1998). Similar studies which investigated the histological components of psoriasis, in comparison with other psoriasiform dermatoses, have used a wide variety of methodological approaches, the most common being the comparison of frequencies of the presence of certain histomorphological elements (elongation of rete ridges, hypogranulosis, presence of Munro or Kogoj pustules and/or epidermal spongiosis); (Beek and Reede, 1977; Singal et al., 2009). In addition, since H&E is perceived as a simple stain which enables researchers to observe apparent differences in

morphology (high sensitivity for identification of structural changes), whilst more specific IHC stains are used to quantify those structural changes seen on H&E, previous histological studies exploring H&E variations between different skin diseases, have only looked into the presence or absence of the various histological characteristics, whilst ignoring potential changes in intensity of those features. The inefficiency of the previous approaches which requires multiple skin samples to power the results and the need for a simple grading system to quantify the various psoriasiform changes have prompted researchers to use more expensive techniques such as the optical coherence tomography, confocal microscopy and power Doppler (Morsy et al., 2010; Gutierrez et al., 2011). This thesis, recognising the need for a simple histological grading method to histologically assess psoriasis and enable simple comparisons between different phenotypes of psoriasis, devised a new histological grading score (**Appendix B**). The intention was to enable a thorough histological examination of PP and PN skin from EOP and LOP samples and then use simple statistics to explore significant differences. The grading system used in this thesis, was based on a weighted histological score devised by Trozak (Trozak, 1994). Trozak's score is simple to use and allows for pre and post-treatment comparisons (Talme et al., 1995). It consists of 12 different histological variables, mainly representing morphological features, each of them graded based on their specificity for psoriasis and disease activity. This thesis' grading score included 18 histological features, each of them scored depending on their severity. In addition, these features were divided into inflammatory and morphological and thus allowed for quantifying the intensity of the inflammatory response and severity of the morphological changes of PP and PN. Simple statistics and plots were used to quantify and depict potential differences.

Previous studies examining PN skin, have shown that compared to normal skin, the capillary blood flow is increased and the presence of abnormal dermal vessels is frequently seen (Klemp and Staberg, 1985; Ross, 1964). In addition, there is some evidence of an aberrant epidermal hyperproliferation of keratinocytes and mild parakeratosis in PN samples (Krueger et al., 1981). The vast majority of the study PN sections simulated normal skin. In line with previous studies, few cases displayed minimal morphological and inflammatory changes, including mild epidermal spongiosis, dilation of blood vessels and perivascular lymphocytic infiltration, in the upper dermis (Van De Kerkhof et al., 1996; Van De Kerkhof, 2007). Upon comparing the PN samples, there were no statistical differences in the three histological scores (THS, TIS and TMS) between the EOP and LOP group.

Interestingly, the assessment of PP samples revealed significant differences in the inflammatory infiltration of LOP sections (**Figure 4.4**). In particular, compared to EOP, LOP samples demonstrated an increased lymphocytic infiltrate in both the epidermis and dermis (mean TIS for EOP=6±2 vs 8±2 for LOP*; t-test, P=0.028*). The analysis revealed an outlier TIS of 14 in a male LOP patient. This patient had mild to moderate psoriasis with a PASI of 5.7 and large, thick plaque psoriasis. When the outlier was removed from the analysis the difference in the mean TIS remained significant. Furthermore, when the analysis controlled for gender, disease duration, extent of psoriasis and clinical phenotype, the aforementioned difference remained significant. This result subsequently led to a higher THS score in LOP compared to EOP (**Table 4.2**). This is the first time that such a difference has been reported and hence warrants further research. The role of lymphocytes in psoriasis has been extensively described in the literature and the intensity of the

cutaneous infiltration has been linked to the severity of the disease and a subsequent drop in the absolute numbers of these cells in peripheral blood (Baker et al., 1984b; Langewouters et al., 2008). Lymphocytes play a key role in the pathogenesis of psoriasis, as they induce pathological immune reactions and alter the epidermal homeostasis of PP skin, which lead in the activation of keratinocytes and other immune cells. Subsequently, the well-described clinical and histological features of psoriasis develop in response to the previous cascade of immune events (Bowcock and Krueger, 2005).

The current results also showed that the mean TMS was not significantly different between EOP and LOP, despite the noted difference in the mean TIS (**Figures 4.3 and 4.5**). The fact that all patients suffered from a mild to moderate disease and were systemic treatment naive, may partially explain the TMS results. It should however mentioned that a linear regression model was employed to control for disease severity and which did not reveal any effect of PASI on TMS. An interesting observation was made, when clinical phenotypes were included in the analysis. It was shown that the thick plaque type exhibited a more severe histomorphological picture compared to the thin plaque phenotype, irrespective of the size of the plaques (small or thin); *(mean TMS for thick plaque=19±3 vs 12±4 for the thin plaque*).

Interestingly, the presence of eosinophils was noted in few of the samples, although there was no indication, from the medical history, for a drug-induced psoriasiform dermatitis. Very little is known about the contribution of eosinophils in the pathogenesis of psoriasis and this knowledge is limited to drug-induced psoriasis. Psoriasis is known to be a Th1 and Th17 driven disease. In contrast, eosinophils are usually activated by type 2 cytokines, released by Th2 cells. Eosinophils have modulatory properties and can influence the inflammatory process in both the epidermis and the dermis of the PP skin (Lundin et al., 1990). Their migration to the epidermis is likely to be related with the presence of IL-5, which is a type 2 cytokine (Kouro and Takatsu, 2009). Previous studies have suggested that despite the presence of type 2 cytokines in psoriasis, they are not thought to be playing a pathogenic role (Uyemura et al., 1993). On the other hand, CCL5 (also known as RANTES; regulated on activation, normal T cell expressed and secreted chemokine) is a chemotactic cytokine which induces recruitment and activation of $CCL5^{+}$ T-cells, mast cells and eosinophils and has been found to play an important role in psoriasis (Herder et al., 2008; Toichi et al., 2006). The RANTES gene is localised to chromosome 17. Psoriasis susceptibility gene 2 or chromosome 17q25 has been shown to be associated with both psoriasis and atopic dermatitis (Capon et al., 2004; Morar et al., 2006). Taken together, the current observations indicate a more active role of the eosinophils in psoriasis and this warrants further investigation.

There are several limitations to this study; the first being, the gender imbalance between the two groups, with EOP having much more male than female, compared to an equal distribution of male and female in the LOP group. To attempt to correct for this imbalance a regression model was employed, with gender as a confounder. The results presented here remained significant, but still the wide 95% CI (0.795-5.466 for the TIS and 0.65-11.71 for the THS) implies that there is either variation in the data or the sample size is small. In addition, all psoriasis subjects had a mild disease (PASI<10 and use of topical treatment for psoriasis). This suggests that that results presented in this thesis may only apply in a specific group of patients with mild to moderate

psoriasis. Using a similar study design, further research is warrant on EOP and LOP patients with moderate to severe and severe disease.

Overall, this is the first study to assess differences in histology between EOP and LOP. A simple grading system was devised for the comparison of a variety of structural and inflammatory parameters. The identification of a higher lymphocytic infiltrate in LOP section from PP skin needs to be further examined and this will be discussed in **chapter 5**. The lack of published data on the topic makes direct comparisons across studies impossible and the repeat of the analysis with a larger sample of sections would be needed to confirm the current results.

5. An immunohistochemical investigation of early and late onset psoriasis

5.1 Introduction

A growing body of clinical and experimental evidence indicates that psoriasis is a T cell-mediated disease (Mueller and Hermann, 1979; Boyman et al., 2004; Gottlieb et al., 1995). Such studies demonstrated clearance of psoriasis after treatment with anti-CD3 and anti-CD4 monoclonal agents, while others showed that skin grafts of PN skin, from psoriasis patients, developed into PP, after transplantation onto SCID mice, which had received direct injection of donor T cells (Weinshenker et al., 1989; Prinz et al., 1991; Wrone-Smith and Nickoloff, 1996). Until recently, it was believed that the inflammatory environment seen in psoriasis was strongly linked to a Th1-induced response (adaptive immunity). Previous research relates *HLA-Cw*06:02* with the Th1-mediated inflammation of psoriasis, as it has been found that its expression is implicated in the activation of peripheral blood $CD8^+$ T-lymphocytes, especially the skin-homing cutaneous lymphocyte-associated antigen-expressing (CLA^+) subset of $CD8^+$ T-cells (Wrone-Smith and Nickoloff, 1996; Tiilikainen et al., 1980; Johnston et al., 2004; McFadden et al., 2009). Recent studies propose that the complex aetiopathogenesis of psoriasis is attributed to a combination of genetic (such as *HLA-Cw*06:02 , IL-12B, IL-23A and IL-23R*) and environmental factors which lead to the dysregulation of both the innate (Th17-pathway) and adaptive (Th1-pathway) immune response (Bowcock and Krueger, 2005; Nestle et al., 2009; Cai et al., 2012).

Immunohistochemical studies have demonstrated that, a large number of T-lymphocytes infiltrate the epidermis and dermis of PP skin, in a non-uniform fashion, with $CD8^+$ T-cells being the main infiltrating T-lymphocyte of the epidermis, while $CD4^+$ T-cells are more abundant in the dermis (Prinz, 1999). Studies on guttate psoriasis have shown that the activation of $CD4^+$, their interaction with APCs and migration to the epidermis, is important for the development of PP, whereas the predominance of $CD8^+$ in the epidermis mainly occurs in chronic lesions (Baker et al., 1984a; Baker et al., 1984b). The HLA-I associated epitopes presented by *HLA-Cw*06:02* molecules (such as type 1 keratins) induce a direct influx of $CD8^+$ T-cells in the epidermis, while HLA-II associated epitopes activate dermal $CD4^+$ T-cells, which stimulate naïve dermal $CD8^+$ T-cells (cross priming of $CD8^+$) and thus trigger their subsequent migration to the epidermis (Gudjonsson et al., 2004). The presence of $CD8^+$ in the epidermis has been associated with the characteristic histological changes seen psoriasis, such as acanthosis and parakeratosis (Gudjonsson et al., 2004; Bovenschen et al., 2005).

The strong association of *HLA-Cw*06:02* with EOP (55-80% of patients) would imply that there is a lower epidermal CD4/CD8 ratio in PP for EOP patients, which is attributed to the aforementioned *HLA-Cw*06:02* -driven tendency of $CD8^+$ to infiltrate the PP epidermis, while $CD4^+$ predominate in the dermis. In contrast, the frequency of *HLA-Cw*06:02* in LOP is 15% and is comparable to that found in the general population (Gujonsson et al., 2002). Thus, potential differences may exist in the pathogenesis of LOP in terms of the landscape of T-cell populations in

PP skin and this warrants further research. As previously outlined in **chapter 1 (section 1.9.4)**, differences exist in LC migration between EOP and LOP. In particular, epidermal LC from LOP PN migrate in response to IL-1β, whereas epidermal LC from EOP PN, do not respond to IL-1β (Shaw et al., 2010).

To explore potential immunological differences between EOP and LOP, an IHC study was undertaken to explore the epidermal and dermal distribution of $CD4^+$ and $CD8^+$ T-cells of PP skin in the two subtypes of psoriasis. No similar studies have been performed previously.

5.2 Objectives

The primary objective of this study was to explore the distribution of $CD4^+$ and $CD8^+$ T cells in PP skin from patients with EOP and LOP. Specific aims were:

- To assess the epidermal $CD8^+$ T-cell counts in PP and PN samples
- To assess the epidermal $CD4^+$ T-cell counts in PP and PN samples
- To calculate the epidermal $CD4^+/CD8^+$ ratio in PP and PN samples
- To examine the intensity of the dermal $CD8^+$ infiltrate in PP and PN samples
- To examine the intensity of the dermal $CD4^+$ infiltrate in PP and PN samples.

5.3 Methodology

The same skin samples were used, from the 31 psoriasis participants of the H&E study (**Chapter 4, section 4.3**). At the Cellular Pathology Department SRFT, 5 mm thick, paraffin embedded sections of PP and PN were cut and stained with anti-CD3, anti-CD4, anti-CD1α and anti-CD8 monoclonal antibodies (**Chapter 2, section 2.2.6**). Stained sections were then coded and randomised in such a fashion that the single assessor (ET) was unaware of the type of psoriasis (EOP or LOP). Positively stained cells in the epidermis were counted per 200X magnification field, across the whole of the epidermis. The positive dermal cells were graded on a 0-3 ordinal scale, with 0 equals normal (0-1 focus of positive cells), 1 minimal (2-4 foci of positive cells), 2 moderate (5-8 foci of positive cells) and 3 severe (>8 foci of positive cells) infiltration.

Initially, epidermal counts were presented as mean values ± SD and comparison between groups was carried out with *one-way ANOVA*. The second level of analysis accounted for confounding variables using a *linear logistic regression*. The primary outcome variables included the mean $CD4^+$, $CD3^+$, $CD8^+$ and $CD1\alpha^+$ epidermal counts, while gender, disease duration, clinical phenotype and PASI were treated as confounders. The type of psoriasis (EOP or LOP) was the main independent variable.

Dermal counts were presented as frequencies and compared for trends using x^2. The statistical significance level was set at 0.05. The statistical analysis was carried out, using SPSS statistical software for Windows, release 20·0 (SPSS Inc., Armonk, NY: IBM Corp U.S.A.).

Blood samples were collected retrospectively, from 21 out of the 31 participants and were HLA-typed, at the Transplantation Laboratory, Manchester Royal Infirmary, Manchester.

5.4 Results

5.4.1 Clinical and demographic characteristics

The study population was the same as that used in the H&E study. Patients aged 50 years old or above and their clinical and demographic characteristics are presented in **Table 4.1, Chapter 4, section 4.4.1**.

5.4.2 Immunohistochemical comparisons of involved and uninvolved skin

The epidermis of PN sections was occasionally infiltrated by $CD4^+$ and $CD8^+$ cells, aligned along the epidermal side of the dermoepidermal junction. These cells were present throughout the entire epidermis of PP skin. In particular, $CD8^+$ cells were predominantly accumulated across the basal and spinous layer of the epidermis, while $CD4^+$ cells were seen primarily in the basal layer. Consequently, the mean epidermal counts from PN sections for $CD4^+$ and $CD8^+$ cells were significantly lower compared to those from PP sections; **Table 5.1**. When the epidermal counts from PN were compared between the two study groups (EOP and LOP), these were not statistically different. However, when the PP sections were examined under the microscope, a prominent $CD8^+$ infiltrate was present in the epidermis of EOP sections, while in the LOP samples, the epidermis was mostly infiltrated with $CD4^+$ cells. This observed difference in the distribution of $CD4^+$ in PP sections, between the two study groups, was statistically significant at the 1% level and is illustrated in **Table 5.1**. The mean difference was mainly driven by an increased intensity of the epidermal $CD4^+$ infiltrate in the LOP group compared to the EOP (mean $CD4^+$ count in EOP=6.7 ± 4.6 vs 15.1±6.2 in LOP; *ANOVA, F=19.15, P<0.001*); (**Table 5.1** and **Figure 5.1**), while the intensity of $CD8^+$ infiltration did not differ between EOP and LOP sections (mean $CD8^+$ count in EOP=19.1±11.1 vs 15.8 ± 7.8 in LOP; *ANOVA, F=0.903, P=0.35*); (**Table 5.1** and **Figure 5.2**). More specifically, the epidermal $CD4^+$ cells of LOP sections were twice that in EOP sections. The epidermal $CD4^+/CD8^+$ ratio of 1.3 in LOP was significantly higher compared to the 0.5 in EOP sections (*ANOVA, F=11.29, P=0.002*); (**Table 5.1**). As the mean epidermal $CD4^+$ data met all normality criteria (*Kolmogorov-Smirnov test*, P=0.2), a *linear regression model* was employed to investigate whether the aforementioned significant difference was influenced by gender, disease duration or severity (PASI) and clinical phenotype. The results of this analysis confirmed the previous *ANOVA* findings. In particular, it was shown that LOP is significantly related to a high epidermal $CD4^+$ count and this relationship is not driven by gender, disease duration, PASI or clinical phenotype (**Table 5.2**).

To further explore the nature of the epidermal $CD4^+$ infiltrate of PP samples, sections were stained with anti-CD3 monoclonal antibodies and the following results were obtained; there was a trend towards significance in the between-group difference of epidermal $CD3^+$ cells, with more $CD3^+$ infiltrating the epidermis of LOP sections (*ANOVA, F=3.805, P=0.061*); (**Table 5.1** and **Figure 5.3B**). At the same time, to exclude the possibility that the $CD4^+$ cells were primarily LC,

the sections were stained with anti-CD1α. The difference in mean epidermal CD1α$^+$ cell count was not statistically significant between EOP and LOP (**Table 5.1** and **Figure 5.3A**).

Cell Types	Positive Epidermal Cells/ 200X field (Mean Values ± SD)				ANOVA P value
	EOP (N=17)		LOP (N=14)		
	PN	PP	PN	PP	
CD3$^+$	-	31.7 ± 17.5	-	42.8 ± 13.3	0.061
CD8$^+$	2.2±2.9	19.1±11.1	1.6±2.8	15.8 ± 7.8	0.58 0.35
CD4$^+$	1.7±1.5	6.7 ± 4.6	1.5±1.6	15.1 ± 6.2	0.67 <0.001*
CD1α$^+$	-	11.1 ± 5.2	-	13.3 ± 6.2	0.30
CD4$^+$/CD8$^+$	3.4±6.8	0.5±0.5	2.4±3.1	1.3±0.8	0.62 0.002*

Table 5.1 Comparison of mean epidermal cells counts of involved (PP) and uninvolved (PN) skin sections, from early onset-EOP (N=17) and late onset psoriasis (N=14) patients.
The table shows the statistical comparisons of mean epidermal counts for CD3$^+$, CD4$^+$, CD8$^+$ and CD1α$^+$ cells of PP and PP, between EOP and LOP groups. The statistical analysis was performed using analysis of variance (ANOVA). The significance level was set at 0.05.*

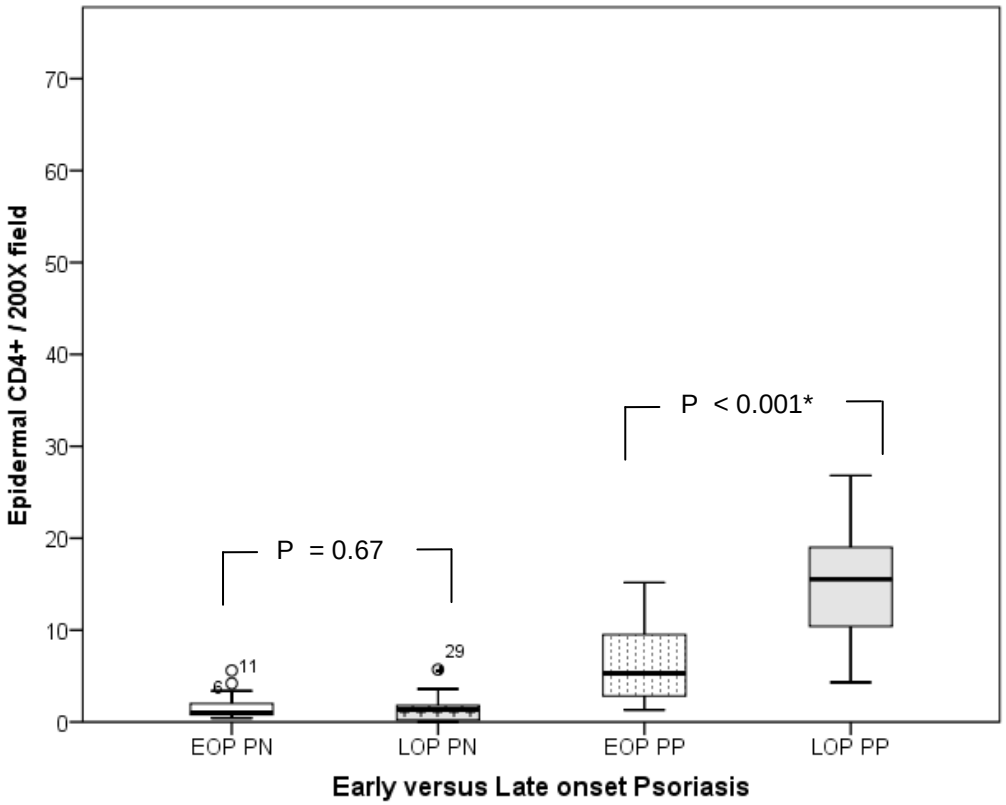

Figure 5.1 Comparison of mean epidermal $CD4^+$ cell counts between skin sections from early onset-EOP (N=17) and late onset psoriasis-LOP (N=14) patients.

The box and whisker diagram illustrates the mean differences of epidermal $CD4^+$ cell counts in involved (PP) and uninvolved (PN) sections from EOP and LOP patients. Subjects 15 and 26 (EOP patients) had a higher than expected $CD4^+$ cell count in PN sections and thus represent outliers 6 and 11 respectively. For the same reason, subject 10 (LOP patient) is presented as outlier 29 in the graph. All outliers were male subjects. The analysis was re-run without outliers and there was no change in the outcome. The significance level is set at 0.05.*

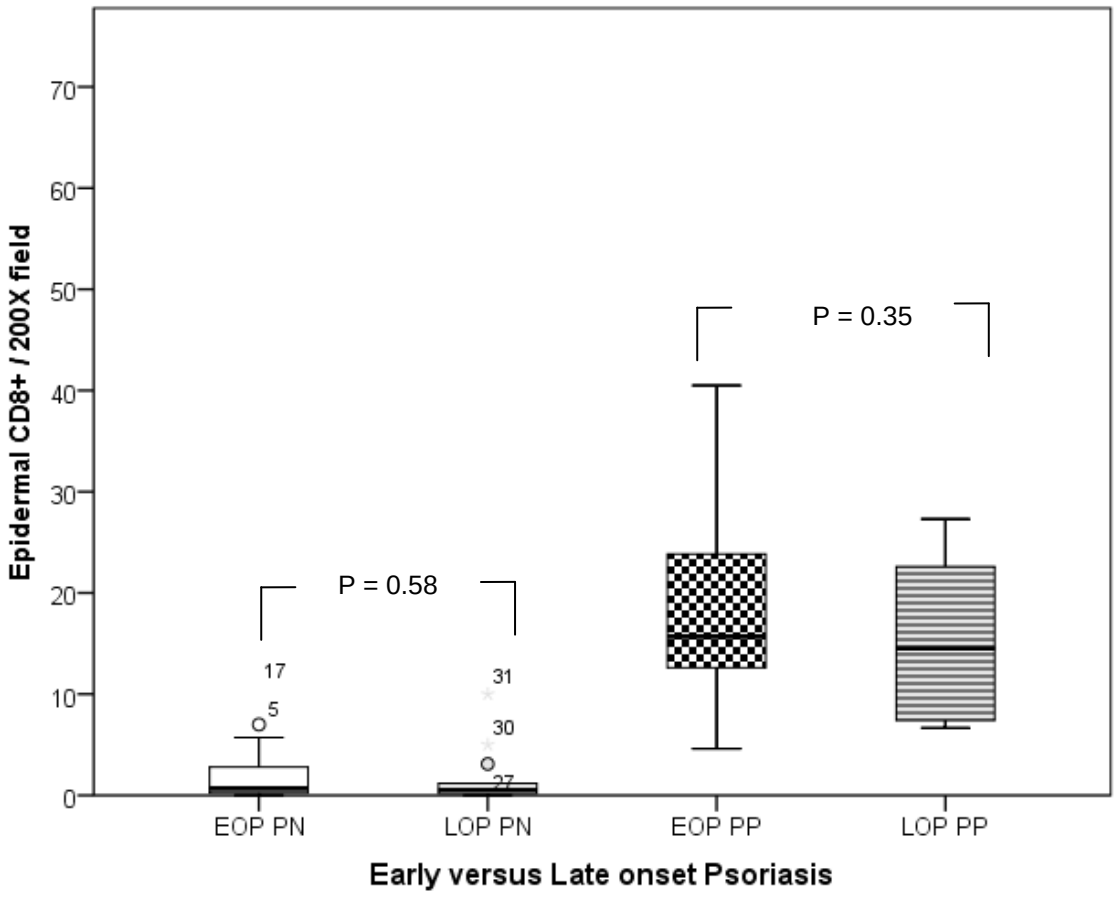

Figure 5.2 Comparison of epidermal CD8$^+$ cell counts between skin sections from early onset-EOP (N=17) and late onset psoriasis-LOP (N=14) patients.

The box and whisker diagram illustrates the mean differences of epidermal CD8$^+$ cell counts in involved (PP) and uninvolved (PN) sections from EOP and LOP patients. Subjects 9 and 16 (EOP patients) had a higher than expected CD8$^+$ cell count in PN sections and thus represent outliers 5 and 17 respectively. For the same reason, subjects 7,8 and 32 (LOP patients) are presented as outliers 27, 30 and 31 in the graph. All outliers apart from 27 (female) were male subjects. The analysis was re-run without outliers and there was no change in the outcome. The significance level is set at 0.05.*

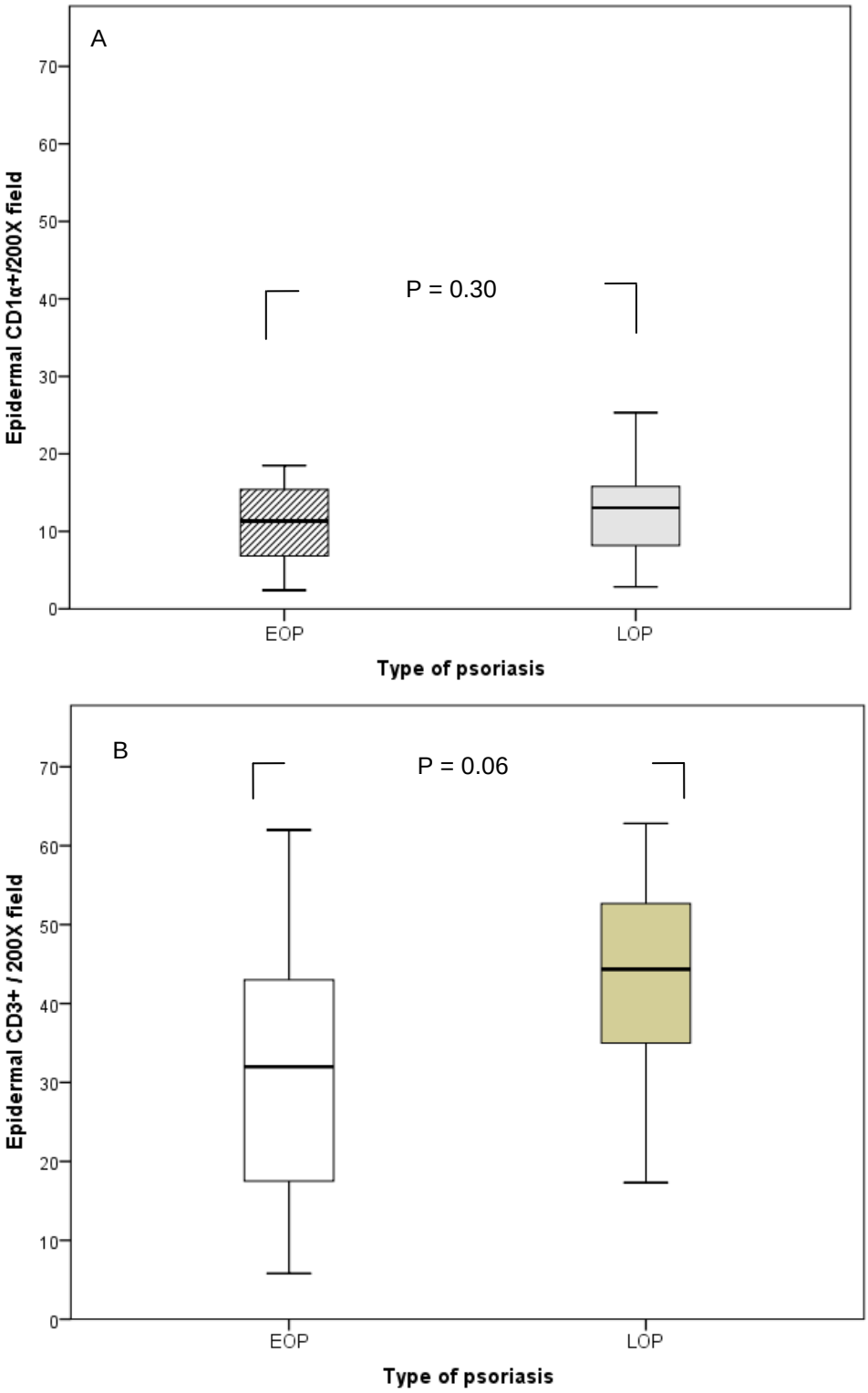

Figure 5.3 Comparison of mean epidermal CD3$^+$ and CD1a$^+$ cell counts in involved (PP) skin sections from early onset-EOP (N=17) and late onset psoriasis-LOP (N=14) patients.

The box and whisker diagrams illustrate the mean differences of epidermal CD1α$^+$ (A) and CD3$^+$ (B) cell counts in PP sections from EOP and LOP patients.

Physical Parameters	Mean Epidermal $CD4^+$ Count		
	Coefficient	P value	95% CI
Type of Psoriasis	11.256	0.003*	4.202-18.312
Gender	0.796	0.722	-3.756 - 5.348
Disease Duration	0.127	0.226	-0.84 - 0.339
PASI	0.324	0.266	-0.262 - 0.911
Clinical phenotypes	0.802	0.767	- 3.206 - 6.780

Table 5.2 The type of psoriasis as the main factor, driving the mean epidermal count difference of $CD4^+$ cells in lesional (PP) skin sections (N=31).

The table demonstrates results from the linear regression model which was employed to rule out potential influences of gender, disease duration, clinical phenotype and disease severity on the outcome (mean epidermal $CD4^+$ count), which, if present, would have lead to spurious results.
CI=confidence interval of the coefficient

Dermis

In the PN sections, the intensity of the dermal $CD4^+$ and $CD8^+$ infiltrates ranged from normal (0) to mild (1), while there was no statistical difference between the two study groups. The PP sections demonstrated a moderate to severe infiltration, with $CD4^+$ showing a higher infiltration of the LOP sections (x^2, *P= 0.049*). Both $CD4^+$ and $CD8^+$ were mainly found in the papillary dermis, perivasculalry and along the rete ridges in close proximity to the dermo-epidermal junction.

Upon examination of the dermal $CD3^+$ and $CD1\alpha^+$, there was again a trend towards significance for a higher dermal $CD3^+$ infiltrate in LOP sections (x^2; *P=0.067*), while there was no difference in $CD1\alpha^+$ cells (x^2; *P=0.79*).

HLA-typing

Results from 14 participants (7 EOP and 7 LOP) were obtained while 6 samples failed to produce results, due to DNA of inadequate quantity. Five out of 7 EOP and 3 out of 7 LOP patients were positive for *HLA-Cw*06:02*. The *HLA-Cw*06:02* status was then compared to the $CD4^+$ infiltrate of the relevant PP section and this was not found to be linked with either a higher or lower $CD4^+$ T-cell count.

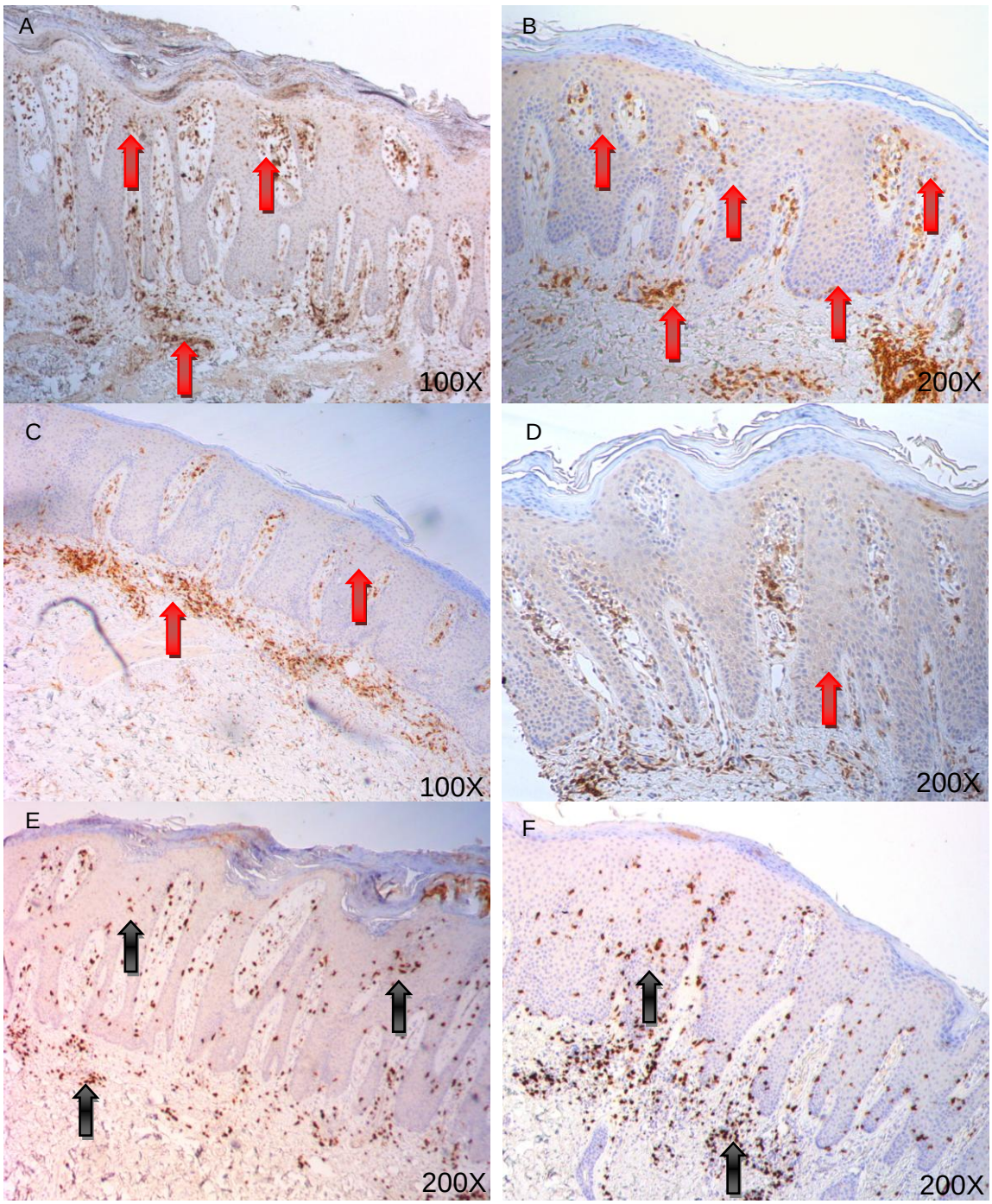

Figure 5.4 Photomicrographs of CD4$^+$ and CD8$^+$ cells from involved (PP) skin sections of early onset (EOP) and late onset psoriasis (LOP) patients.

Photomicrographs from LOP (A,B) and EOP (C,D) sections showing the distribution of CD4$^+$ cells in the epidermis and dermis (red arrows). The distribution of CD8$^+$ infiltrate (black arrows) in PP sections is illustrated in photomicrographs E (LOP) and F (EOP).

5.5 Discussion

The results of this study show statistically significant differences in the distribution of T-cell subtypes between EOP and LOP. Compared to EOP, the LOP patients showed twice as many $CD4^+$ cells in the PP epidermis (mean $CD4^+$ count in EOP=6.7 ± 4.6 vs 15.1±6.2 in LOP; *ANOVA, F=19.15, P<0.001*); (**Figure 5.4A-D**). Subsequently, this led to a significantly higher $CD4^+/CD8^+$ ratio in the LOP group (1:2 in EOP vs ~1 in LOP; *ANOVA, F=11.29, P=0.002)*. In addition, dermal $CD4^+$ counts were higher in the LOP group (x^2*, P= 0.049*). This is in line with the observation made in the H&E study, where the intensity of the lymphocytic infiltrate was found to be significantly higher in the LOP sections *(*mean TIS for EOP=6±2 vs 8±2 for LOP*; t-test, t=2.32, P=0.028)*. The immunophenotype of these $CD4^+$ cells, was ascertained as being T-cells, since the $CD1\alpha^+$ cell counts were comparable between the two groups (mean $CD1\alpha^+$ in EOP=11.1 ± 5.2 vs 13.3 ± 6.2; *ANOVA, F=1.11, P=0.30)*, while the $CD3^+$ cells showed a higher infiltration in the LOP group (mean $CD3^+$ in EOP=31.7 ± 17.5 vs 42.8 ± 13.3 in LOP; *ANOVA, F=3.81, P=0.061*). In addition, *HLA-Cw*06:02* was not found to correlate with the observed $CD4^+$ influx in LOP. All biopsies were taken from chronic lesions, which suggests that a high epidermal influx of $CD4^+$ T-cells is not exclusive to early phase psoriasis lesions (Onuma, 1994). Influx of $CD4^+$ cells may also play a crucial role in the development and maintenance of the inflammatory process in LOP and unlike *HLA-Cw*06:02*$^+$ EOP, this may not be linked to *HLA-Cw*06:02*.

It should be noted that there was a gender imbalance between study groups, with 80% of EOP patients being male, whilst this was not the case for the LOP group, where male to female ratio was 1:1. At the same time, it was noted that LOP patients had a shorter disease duration compared to EOP. Moreover, all patients had a low PASI and were using topical agents to treat their psoriasis, which implies that their disease was mild. As the three aforementioned key confounders might have influenced the *ANOVA* results, a regression model was employed to reveal any influences on the outcome (mean epidermal count) from these confounding variables. The regression results confirmed the presence of significant differences in the mean epidermal $CD4^+$ counts between study groups and hence, it can be assumed that the current results show actual differences in pathomechanism between EOP and LOP, whilst are not influenced by obvious physical parameters such as gender, disease duration and severity or clinical phenotype.

Although epidermal hyperproliferation and dyskeratinisation have been linked with an influx of $CD8^+$ cells in the epidermis, both EOP and LOP examined sections were found to have an equal incidence of acanthosis and parakeratosis (**Chapter 4, section 4.4.3.1**), despite the difference in $CD4^+$ infiltration (Conrad et al., 2007; Deguchi et al., 2001). This suggests that other factors may play a part in epidermal remodelling in psoriasis. To further look into those previous findings of **chapters 4 and 5**, it would be interesting to immunhistochemically explore potential differences in the distribution of Ki-67^+ cells (which are indicative of epidermal proliferation) and the K-10+ cells (which indicate normal keratinisation) between the two psoriasis groups.

The current methodology had limitations. The IHC staining of cells is often suboptimal, which makes the cell counting difficult. Confocal microscopes and fluorescence activated cell sorters may be more accurate. In addition, the IHC antibodies are not T-cell specific. Moreover,

some IHC stains are more intense than the others ($CD8^+$ and $CD3^+$ vs $CD4^+$), which results in variable sensitivity and specificity. Furthermore, the mild to moderate disease course of psoriasis of all volunteers, on the day of biopsies, may have influenced the outcome, as they were no data on patients with severe disease. The small sample size is also another limiting factor for generalising the current results and this is shown by the wide 95% confidence interval. Despite the abovementioned shortcomings, the analysis of the results in this study was carried out by the same observer (ET) and the IHC staining was performed in a standardized manner, by an automated machine, while the analysis was based on a multivariate model, which accounted for the effect of relevant confounding factors, including disease severity, disease duration and clinical phenotype. The previous make this study's methodology suitable for the investigation of the different T-cell subsets. Finally, another disadvantage of this study is the limited information on the *HLA-Cw*06:02* profile of the study patients.

The current results suggest that there are important immunological differences between the two groups, particularly a $CD4^+$ epidermotropism in LOP (**Figure 5.3**). The increase in the epidermal $CD4^+$ infiltration and subsequent imbalance in the ratio of $CD4^+/CD8^+$ in the LOP group is a differentiating factor between the two subtypes of psoriasis. Future immunological comparisons are warranted. This can be achieved by studying the different subtypes of T-cells. These include $CD4^+$ cells, including the different subtypes of Th cells (Th1 and Th2), as well as Treg cells. Two other types of T-cells might also be important to investigate: IL-17 producing T-cells and NKT cells. In addition, further immunological differences can be investigated, between the two groups, by examining for differences in the expression of soluble mediators, especially that of IL-1β and TNF-α. Furthermore, it would be interesting to ascertain whether these $CD4^+$ cells express both the a_1b_1 and $\alpha_E(CD103)\beta_7$ phenotype (**chapter 1, section 1.5.2**) and whether the majority is capable of producing type 1 cytokines (IFN-γ, TNF-α, IL-2). Perhaps even more intriguing would be future studies to speculate whether anti-psoriatic treatments targeting activated T-cell subtypes, are effective in both EOP and LOP psoriasis or whether there are any differences.

6. General Discussion

6.1 Background

In 1985, Henseler and Christophers, categorized psoriasis according to age of onset (EOP, age of onset ≤ 40 years of age and; LOP, age of onset > 40 years of age) and remarked that these two categories also differed in their clinical and genetic characteristics (Henseler and Christophers, 1985; Henseler and Christophers, 1998). Recent studies on this topic have both confirmed the previously described clinical and genetic differences between EOP and LOP as well as identified additional features such as distinct immunological differences between these two subtypes of psoriasis (Gudjonsson et al., 2006a; Gudjonsson et al., 2002; Shaw et al., 2010; Ferrandiz et al., 2002; Gupta et al., 1996). More specifically, it has been shown that EOP patients experience a more severe disease course, usually requiring 2nd and 3rd line treatments. Guttate psoriasis, often triggered by streptococcal pharyngitis, is more commonly seen in EOP patients (Gudjonsson et al., 2002). In addition, the eruptive nature of EOP is often associated with high psychosocial impact, although recent findings suggest the opposite (Kotrulja et al., 2010; Remröd et al., 2013b). On the other hand, LOP seems to be a less severe skin disease than EOP, which responds to topical agents and phototherapy (Ferrandiz et al., 2002). Some studies show a higher prevalence of PsA with LOP, whilst others link LOP to T2DM and metabolic syndrome (Armesto et al., 2012; Gudjonsson et al., 2006b).

Despite those described differences, it is, however, still unclear, whether the underlying pathomechanisms that lead to either EOP or LOP are different. Current data show that the clinical differences of EOP and LOP may be driven by genetic variation. In particular, patients with EOP are more likely to be *HLA-Cw*06:02* positive and have a family member who also suffers from psoriasis (Henseler and Christophers, 1985; Gudjonsson et al., 2002). New data show that EOP is also linked with polymorphisms in *ERAP1*, *TNFA promoter*, *IL-12B* and *IL-23R* genes (Lysell et al., 2013; Reich et al., 2002; Smith et al., 2008a). Late onset psoriasis, however, is not linked with *HLA-Cw*06:02 ,* and is more sporadic (Stuart et al., 2002). Recent evidence suggests that LOP is possibly genetically linked with polymorphisms in the *IL-1B* gene (Reich et al., 2002). One study, looking at immunological differences between EOP and LOP, demonstrated that epidermal LC from PN of EOP did not migrate in response to either IL-1β or TNF-α, whilst LC from PN of LOP migrated in response to IL-1β (Shaw et al., 2010).

The overall aim of the work presented in this thesis was to perform a careful and thorough examination of the clinical, histological and immunological differences between EOP and LOP thereby enhancing our understanding of these two forms of psoriasis. Clinical and psychological differences were examined using prospective and retrospective clinical data from 340 psoriasis patients (**chapter 3**). Variations in histology and immunohistology were examined in skin biopsies from 31 psoriasis patients (**chapters 4 and 5**).

6.2 Summary of findings

The results from the data generated in this thesis provide further evidence that EOP and LOP should be considered as distinct entities.

6.2.1 Clinical, psychological and inheritance differences between early and late onset psoriasis

Chapter 3 explores clinical, heritable and psychological differences between EOP and LOP patients. The data show that EOP and LOP have variance in clinical presentation and that they are inherited in different patterns. More specifically, and in line with previous reports, EOP patients had a more severe and active disease compared to LOP, characterised by an eruptive phenotype, often triggered by URTIs and requiring biologic treatment, whereas, LOP patients presented with a milder clinical phenotype, usually responding to phototherapy (Gudjonsson et al., 2002; Ferrandiz et al., 2002; Henseler and Christophers, 1985; Harari et al., 2012). Severity of psoriasis is not merely represented by the extent of skin involvement, but is also dictated by psychosocial disability, as well as therapeutic responsiveness. Therefore, it is important to acknowledge that this study used a severity tool which encountered both physical and psychological disability.

This significant difference in therapeutic management could potentially be explained by the observed, higher incidence of thin-plaque psoriasis phenotypes (small-thin and large-thin) in LOP and thick-plaque in EOP patients, as studies have demonstrated that small, thin-plaque psoriasis responds well to 1st line treatments (Rakkhit et al., 2009; Zanolli, 2004). This is important to acknowledge as future guidelines on the management of the disease should consider such findings and potentially utilise different therapeutic strategies for EOP and LOP. A larger cohort is required to confirm the results presented here and further explore whether the good response of LOP to 1st line treatments is driven mainly by the thickness of psoriasis plaques.

There is contradictory evidence on whether nail changes are more prominent in LOP patients. Some studies have demonstrated a high prevalence of nail psoriasis and PsA in patients with LOP and negative *HLA-Cw*06:02* (Mallbris et al., 2005; Gudjonsson et al., 2006a). On the other hand, other studies have shown that LOP patients have a lower likelihood in developing nail changes compared to EOP (Henseler and Christophers, 1985; Stuart et al., 2002; Ferrandiz et al., 2002). The fingernail data showed that PsA and nail psoriasis were equally present in both study groups, but it was observed that specific clinical features, such as onycholysis and subungual hyperkeratosis (nail bed changes), were more frequent in LOP patients. This is consistent with findings published by Gudjonsson and co-workers, who also observed that apart from nail bed changes, LOP patients were more likely to have nail matrix abnormalities, such as pits (Gudjonsson et al., 2006a). Data from toenails were not included in this analysis, as these are more prone to fungal infections which clinically resemble nail psoriasis. This thesis also confirmed

that the number of affected nails is positively associated with the presence of PsA in both EOP and LOP patients.

Similar to previous studies, guttate, erythrodermic and pustular psoriasis were more frequent in EOP patients, while PPP and scalp psoriasis were more often seen in LOP patients (Fan et al., 2007; Gudjonsson et al., 2006a). These studies have associated the aforementioned variations with the presence or absence of *HLA-Cw*06:02* . In this thesis, there were no data on the *HLA-C* status of questionnaire study participants and this is a weakness of the current study and an important consideration for future work.

As presented in other studies, compared to EOP, LOP is more sporadic and hence LOP patients have a lower likelihood of having a relative with psoriasis (Ferrandiz et al., 2002; Henseler and Christophers, 1985; Gudjonsson et al., 2002; Holgate, 1975). In line with these studies, it was demonstrated that patients with first and/or second degree relatives with psoriasis were less likely to suffer from LOP and it was, thus, confirmed in this thesis, that LOP is more of a sporadic disease, potentially triggered by environmental changes. Furthermore, this study was the first to investigate differences in the inheritance pattern of EOP and LOP and the first to show that patients with EOP parents were less likely to develop LOP than EOP. This finding is consistent with previous research, as it confirms the familial nature of EOP and also may suggest that EOP and LOP have a different genetic basis. Early onset psoriasis has previously been linked with *HLA-Cw*06:02* which is considered a major genetic risk factor, while a major genetic link to LOP has yet to be identified (Allen et al., 2005; Szczerkowska-Dobosz et al., 2007).

Patients with chronic, immune-mediated diseases have a higher risk of developing other autoimmune and concurrent comorbid conditions (Robinson et al., 2006). Psoriasis has been linked with various comorbidities such as inflammatory bowel disease, metabolic syndrome, IHD and T2DM (Armstrong et al., 2012; Makredes et al., 2009; Gelfand et al., 2006). Many studies have demonstrated that psoriasis is linked to T2DM, in such a manner that among psoriasis patients there is a 60% higher prevalence of T2DM compared to healthy individuals, with younger patients with severe disease being more predisposed to T2DM (Armstrong et al., 2012). This link is potential driven by similar pathomechanisms, such as angiogenesis, oxidative stress and expression of certain genetic loci (Kim and Kim, 2005; Kimball et al., 2008; Quaranta et al., 2009; Tekin et al., 2007). In this study, investigations of differences in associated psoriasis co-morbidities showed that LOP patients were approximately three times more likely to develop T2DM, compared to age-matched controls, while, in the EOP group, no such association was found. Furthermore, EOP subjects were less likely to develop AIT compared to controls. This, however, was found to be non-significant in LOP subjects. Other studies have demonstrated a link between T2DM and LOP but their analysis did not account for various confounders which are strongly correlated with T2DM, such as age, gender, disease duration, obesity, dyslipidemia and disease severity and hence could have influenced their outcome, resulting in spurious results (Armesto et al., 2012; Mazlin et al., 2012). To account for classically linked variables of T2DM, this study adjusted for age, gender, BMI, disease duration, history of diagnosed HTN, dyslipidemia and severity of psoriasis, to minimise confounding. The current findings raise the possibility of genetic and pathophysiological associations between T2DM and LOP, as well as therapeutic implications for patients with LOP. These results on T2DM also suggest that LOP patients may need to be monitored for certain

comorbid conditions from onset, in a prospective manner. Previous work has indicated that disease severity seems to be linked with T2DM (Armstrong et al., 2012), but the results presented here were unable to identify such a link. This discrepancy may be explained by the fact that a different severity tool was used in this thesis to assess disease severity, compared to most studies, that base their results in PASI. The clinical prediction tool of this study, used to evaluated disease severity, although measured clinical and psychological severity, as well as therapeutic management, it has not been validated, and yet remains a study specific, research instrument, not applicable for clinical use. The positive aspect of this assessment tool is that it encounters the clinical severity at a certain time-point (study visit), along with the therapeutic regiment (1^{st}, 2^{nd} or 3^{rd} line treatment) and the psychosocial impact of the disease on patient's life. It is therefore an interesting addition to usual severity tools, and further research is warrant to validate its clinical use.

The findings relevant to AIT and EOP are intriguing and warrant further study. Few studies, exploring links between AIT and psoriasis have identified any direct link, apart from one italian study (Wu et al., 2012; Gul et al., 2009; Antonelli et al., 2006). Autoimmune thyroiditis shares genetic risk factors with psoriasis (*cytotoxic T-lymphocyte antigen 4 or CTLA-4, CD40, PTPN22, HLA-DR4**); (Zervou et al., 2011; Ban, 2012; Jullien and Barker, 2006; Smith et al., 2008b). Some of these genes have also been linked to EOP (Smith et al., 2008b; Henseler and Christophers, 1985). Recent data also suggest that the aetiopathogenesis of AIT is linked to dysfunctions of $CD4+CD25+^{high}$ T_{reg} cells, as is the case in psoriasis (Bossowski et al.; Nestle et al., 2009; Sugiyama et al., 2005). Given the central contribution of the *Forkhead box P3 (FoxP3) gene* to T_{reg} "behaviour", polymorphisms in *FoxP3* have been directly linked to these cells' dysfunction (Sakaguchi et al., 2006). Polymorphisms in *FoxP3* have been associated with both psoriasis and AIT (Ban, 2012; Sugiyama et al., 2005). This hypothesis, as well as confirmation of the current results, is yet to be explored.

The psychological impact of psoriasis is well-recognised and can lead to patients having physical, interpersonal and social impairment (da Silva et al., 2006; Fortune et al., 2002; Fortune et al., 2005; Griffiths and Richards, 2001). Early onset psoriasis has been associated with a greater psychosocial co-morbidity, mostly deriving from the more extensive and eruptive nature of EOP, the younger age of these patients, as well as their marital and employment status. Furthermore, EOP patients are more likely to be single (single individuals are expected to have a higher psychosocial impact compared to married ones), and experience high stress at work (the stressful working environment can be a major factor of distress for patients with psoriasis, compared to those who stay at home); (Ferrandiz et al., 2002; Leary et al., 1998). Moreover, traits of psychological predisposition to maladaptive responses in stressful events and pessimistic personality traits are associated with EOP (Remröd et al., 2013a). On the other hand, LOP has traditionally been thought to be less stigmatising, with a better QoL and a lower incidence of mood disorders, compared to EOP (Gupta et al., 1996; Henseler and Christophers, 1985). Contrary to previous reports, the results presented here suggest that both EOP and LOP have a moderate impact on QoL of patients. In addition, depression was not linked to either study group. Conversely, one study from Croatia demonstrated that LOP patients had significantly higher levels of depression, which was attributed mainly to the sudden change of these patients' self-image later in

life, and status of interpersonal relationships, as well as the subsequent lack of coping mechanisms for managing their condition (Kotrulja et al., 2010). In addition, these patients were found to have a specific "neurotic" profile, featured by high levels of depression, hysteria and hypochondriasis, which made them potentially more prone to maladaption to environmental stressors. Although this thesis did not identify specific personality traits, nor any link between depression and any of the study groups, a strong link was demonstrated between high levels of anxiety and LOP patients. Anxiety is a mood disorder, like depression, and hence partially confirms the hypothesis, that LOP patients might be more vulnerable to external stressors (Kotrulja et al., 2010). Finally, it was shown in this thesis that some clinical phenotypes, with highly impaired QoL and increased interpersonal disability due to the location of the skin lesions (such as PPP and scalp psoriasis), were more prevalent in the LOP group and this may partially explain the increased levels of anxiety in this group. Moreover, recent evidence indicates that psychological distress is directly linked to response to treatment. A UK study showed that psoriasis patients undergoing PUVA were less likely to respond to therapy, if they suffered from high-levels of anxiety and worry, compared to low-level worry groups (Fortune et al., 2003). This is important as this study was able to show that LOP patients were more likely to receive phototherapy instead of other systemic treatments. As this group of patients was identified as high-level anxiety group, it would be interesting to know whether this influences their response to treatment and if so, whether these patients would benefit from adjunctive psychological intervention before and after treatment. Finally, it should be mentioned that the psychometric tools used in this thesis were the DLQI, HADS, PSWQ and BD-II, which are commonly used in clinical practice with good results. The DLQI is a common QoL measurement, frequently used in Dermatology clinics, whilst HADS, PSWQ and BD-II are often used in hospital patients to assess psychological distress and mood disorders, as they exhibit high internal consistency and stability (test-retest interclass correlation).

6.2.2 Histological and immunological differences between early and late onset psoriasis

Chapter 4 discussed histological differences in H&E stained skin sections of PP and PN, from EOP and LOP patients. Several histomorphological and inflammatory parameters were examined. Although there were no significant morphological differences between the two subtypes of psoriasis in both PP and PN sections, the current findings identified a higher inflammatory infiltrate to predominate in LOP, PP sections. This was found to be significantly different to EOP, PP samples. To my knowledge, this is the first study to explore histological differences between these two subtypes of psoriasis, and the current findings are hence novel.

Histologically psoriasis is defined as a psoriasiform dermatitis, which, similarly to its clinical picture, is highly heterogeneous. Previous literature exploring differences among different clinical phenotypes of psoriasis, or psoriasiform dermatoses, has mainly focused in identifying histomorphological variations (Beek and Reede, 1977; Gutierrez et al., 2011; Krengel et al., 1998; Morsy et al., 2010; Singal et al., 2009; Trozak, 1994). This is the first study which distinguishes the histological features of psoriasiform dermatitis into morphological and inflammatory. Additionally,

individual features were scored on a semi-quantitative scale, based on the presence and intensity. In this way, it became possible to thoroughly examine and compare a wide variety of parameters and explore differences in both structural and inflammatory components. It is important to note that different phenotypes of psoriasis show overlapping histological features and hence, the lack of statistically significant differences in the morphology of the PP skin in the EOP and LOP groups was not a surprise. In addition, an interesting observation was made, when the presence and severity of structural features was examined between the different clinical sub-types of plaque psoriasis. More specifically, as expected, it was shown that lesions from patients with thick plaque psoriasis had more profound morphological changes, compared to those from thin plaque psoriasis, and was irrespective of the size of the plaques (large or small). At the same time, it should be noted that biopsied patients presented with equal proportions of clinical sub-types and hence, it is less likely that the findings on the morphological parameters of skin sections have been influenced by this parameter. On the other hand, statistically significant differences were identified when the inflammatory components of the H&E sections were examined, with LOP showing a more intense infiltration of immune cells, irrespective of gender or disease duration.

An IHC study was carried out, using the same skin samples (**chapter 5**). Skin sections were stained for CD4 and CD8, which represent the main surface markers of lymphocytes, implicated in the pathomechanisms of psoriasis. The data presented in this chapter, discuss immunological differences between EOP and LOP. These differences include different infiltration patterns of the PP skin by $CD4^+$ and $CD8^+$ cells and a subsequent imbalance in the epidermal $CD4^+/CD8^+$ ratio (1:2 in EOP versus ~1 in LOP). In particular, compared to EOP, $CD4^+$ cells were more abundant in the epidermis of LOP sections, whilst a similar pattern was present in the dermis of LOP samples. To identify the immunophenotype of these $CD4^+$ cells, sections were stained for CD3, which is a general marker for T-lymphocytes and CD1a, which is specific to LC. Results from this analysis demonstrated that the $CD4^+$ cells infiltrating the LOP epidermis were mainly T-lymphocytes and not epidermal LC ($CD4^+CD1\alpha^+$ cells), as epidermal $CD1\alpha^+$ cells were equally distributed in EOP and LOP, PP sections, whilst, as mentioned above, epidermal $CD4^+$ cells were more abundant in LOP, PP sections. This striking new finding suggests distinct immunological differences between EOP and LOP. It is well-established that T-lymphocytes play a key role in the immunopathogenesis of psoriasis, with $CD4^+$ T-cells initiating and $CD8^+$ maintaining the inflammatory response (Baker et al., 1984b). The contribution of *HLA-Cw*06:02* in inducing the infiltration of epidermis by $CD8^+$ can lead one to expect significant differences in the distribution of $CD8^+$ between EOP and LOP, instead of the observed differences in $CD4^+$ T-cells. This thesis was not able to identify such differences in the $CD8^+$ infiltration of both the dermis and epidermis. These data imply that *HLA-Cw*06:02* is not the sole factor for the migration of $CD8^+$ from the dermis into the epidermis.

The observed epidermotropism of $CD4^+$ T-cells in LOP, may suggest that these cells have effector properties and hence not only trigger but also sustain the chronic inflammation. It may now be reasonable to postulate that these immunological variations are driven by differences in the cytokine microenvironment and cell signalling pathways of EOP and LOP. One previous study looked at differences in the antigen recognition by T-cells between EOP and LOP (Schmitt-Egenolf et al., 1991). This study explored variations in the α and β chain of TCR. Although the authors were

unable to show differences in the use of TCR, they demonstrated a higher epidermal $CD3^+$ infiltrate in the LOP PP. This is in line with the current results and supports this thesis' findings. Another study, looking at immunological variations between EOP and LOP, demonstrated that the migration of epidermal LC from PN skin of LOP patients is different from that of EOP patients (Shaw et al., 2010). Epidermal LC migration requires signalling from both IL-1β and TNF-α. Results from this study showed that LC from LOP, PN sections were able to mobilise out of the epidermis, upon direct intradermal injection of IL-1β, whereas the same cells, from EOP, PN sections, were unresponsive to both exogenous IL-1β and TNF-α. Although these results have not been confirmed in PP sections, demonstrated clear differences in production, bioactivation and/or signalling of IL-1β between these two subtypes of psoriasis. As mentioned previously in this paragraph, this may be triggered by a different local cellular microenvironment. On these grounds, the distinct cytokine profile seen in PN skin of LOP and the resulting behaviour of epidermal LC may trigger the profound mobilisation of $CD4^+$ T-cells from the dermis to epidermis. Further research is now warranted to confirm these findings and explore potential underlying mechanisms for the observed $CD4^+$ epidermotropism.

6.3 Limitations of the study

It is important to critically evaluate the overall study design of this thesis and acknowledge the various weaknesses. Certain limitations of the three different studies of this thesis have also been discussed throughout the thesis (**chapters 3, 4, 5**).

6.3.1 Questionnaire study

The design and analysis of the questionnaire study were based on similar studies on the topic (Henseler and Christophers, 1985; Ferrandiz et al., 2002; Gudjonsson et al., 2006a; Gudjonsson et al., 2002). These studies were either on prospective or retrospective cohorts, using questionnaires or computerised medical records. More specifically, in addition to physician-assessed questions and clinical examination, the prospective cohorts also used patient-reported questionnaires to assess the clinical and demographic characteristics, as well as the psychosocial disability. Various statistical tests were employed to assess associations and significant differences between EOP and LOP. As standard, the endpoint assessment of significant associations included *x^2 or Fisher's exact test,* while *binary logistic regression* was performed to confirm the significant associations and adjust for confounders. In this thesis, a case-control study was conducted, in which, by default, the target population is selected in such a way so that the prevalence of a disease in that sample is not reflective of the prevalence of the disease in the population of interest and hence, risk ratios are not valid. Odds ratios in this case are the most appropriate way to estimate probability of disease.

A common type of bias in many questionnaire studies is that of selection and information. It could be argued that volunteers who participated in the questionnaire study (**chapter 3**) differ

with regards to disease status from those who did not participate and hence, produce spurious results. However, subjects were recruited from multiple channels including both physician and self-referrals, in addition to advertising and direct mailing, so as to reduce any problems of generalisation (selection bias). It should be mentioned that the vast majority of patients were Caucasian, which means that the findings presented here may apply only to this ethnic population. In addition, the study sample was not as large as other studies and although it was powered to demonstrate differences in the frequency between certain, known comorbidities (T2DM, IHD, Crohn's disease, atopy), it may potentially have not allowed for detection of relationships between rare diseases and psoriasis. Moreover, it should be noted that psoriasis patients have a slightly higher incidence of inpatient visits compared to controls and hence these patents are more likely to be diagnosed with a comorbidity. The strength of this study is that electronic patient records were used, which provided access to comprehensive information from primary care integrated with secondary care, where controls would be screened and monitored for common conditions, such as HTN, obesity, dyslipidemia and T2DM.

To avoid any interviewer bias, the same trained interviewer (ET) was used throughout the study to record and interpret the information received from the study participants. Careful wording was also used to avoid misleading questions. One could also argue that recall bias from inaccurate recall of past events may be a major disadvantage in a questionnaire study. Although the current study cannot exclude that such a bias may have inflated the current findings, checks against medical records were undertaken, to minimise this form of bias. In addition, questions on medical history were made in both A and B part of the questionnaire and answers were cross-checked by the same researcher. It should be noted that part A was completed by the interviewer, whereas part B by the study subject.

Finally, this study design was based on data collected in a prospective and retrospective manner. The examination of the first 100 patients was carried out using a prospective survey (study questionnaire), where facts were confirmed in a detailed and consistent manner, by one investigator. The main disadvantage of the retrospective study is that there was no control over how the original data were collected. The second group can be considered a retrospective survey of psoriasis clerking proformas, which were not designed for this study and therefore, some parameters lacked data. The positive aspect though is that these proformas were designed by academic staff of the psoriasis clinic so as to include detailed demographic and clinical information of newly referred patients. In addition, the study questionnaire was based, to a significant degree, on this clerking proforma. Finally, it is important to acknowledge that the retrospective data mainly come from a hospital-based population referred to tertiary care, although the prospective data were collected from multiple sources.

6.3.2 Histology and immunohistochemistry study

The design of the biopsy study was similar to previous relevant studies (Cameron et al., 2002; Watson et al., 1998; Beek and Reede, 1977; Trozak, 1994; Singal et al., 2009; Morsy et al., 2010). There was a male predominance in the EOP group of the biopsy study, which, although when adjusted for as a potential confounder, it did not demonstrate any effects on the outcome

results, still may add some discrepancy in the findings presented here. Criticism can also be presented concerning the differences in the disease duration, as well as previous and current use of medications, which may have influenced the study findings. There is no clear evidence in the existing literature that disease duration significantly affects the $CD4^+/CD8^+$ infiltration of psoriasis plaques. Although difficult to control for disease duration, there was an attempt to recruit subjects with minimal or similar use of non-psoriasis medications. Moreover, all study subjects were systemic treatment naive. Finally, all biopsied lesions had similar clinical characteristics, were chronic phase lesions (>6 months) and were taken from sun-protected upper buttock. Still the likelihood of spurious results driven by differences in the chronicity, thickness and size of the plaques, cannot be excluded, as these ranged from patient to patient. As aforementioned, the staining technique used may have been suboptimal, but the fact that an automated staining protocol was used, instead of manual, makes the methodology robust to outliers.

Moreover, all biopsied patients had a mild to moderate skin disease (mean PASI<10), well-controlled with topical treatment. The previous indicates that the current results may only refer to a particular group of patients and additional research is required to investigate similar histological and immunological differences in moderate to severe and very severe psoriasis phenotypes. However, it should be noted that the current analysis model included PASI as a confounding factor and was unable to detect any links among the outcome variables (TMS, THS, TIS, $CD4^+$ and $CD3^+$ counts and $CD4^+/CD8^+$ ratio) and disease severity.

The statistical model used was based on a two level analysis. The first level included simple comparisons of means or ranks, depending on the nature of the outcome variable, regardless of the various confounders presented above. The second level comprised a regression model which allowed for confounding variables to be included in the analysis. This way significant interactions among confounders and/or outcome/independent variables were identified, which may have influenced the original relationship between outcome and independent variables of the first level of analysis and hence lead to spurious results.

Another limitation of this study was the lack of data on the HLA-C status of all subjects. Based on the previous literature (Krueger and Bowcock, 2005), it was hypothesised that LOP patients, who are more likely to be *HLA-Cw*06:02* negative, were expected to show a CD4/CD8 imbalance in the PP skin. The ethics approval ("Skin procurement study") used in the first steps of this study did not allow for collection of blood specimens. This was though feasible during the second year of the PhD thesis, with the use of the new ethics application ("Immunogenetics of psoriasis") and thereafter, recruited patients were also asked to give blood for further genetic analysis. Despite the effort to obtain blood from all patients (under both ethics' approvals), only 21 (out of 32) samples were collected. In addition, a laboratory problem with the extraction of DNA, resulted in producing HLA results for only 14 patients (7 EOP and 7 LOP). This way it was not possible to provide substantial evidence on whether the presence of absence of *HLA-Cw*06:02* was correlated with the observed $CD4^+$ epidermotropism of LOP samples.

6.4 Overall conclusions

In this thesis, the key aim was to investigate differences between two subtypes of psoriasis, EOP and LOP, which are based on the age at disease onset. The data herein demonstrate significant clinical (**Chapter 3**), psychological (**Chapter 3**), histological (**Chapter 4**) and immunological differences (**Chapter 5**) between patients who develop psoriasis in a younger age compared to those who develop the condition in their 5^{th} decade (**Table 6.1**). In particular, it was demonstrated that EOP is a clinically more severe, eruptive skin disease, more frequently requiring 3^{rd} line systemic treatment, and has a strong familial background and thus genetic component compared with LOP. On the other hand, LOP effectively responds to first (topical agents) and second line treatments (phototherapy and systemic non-biologic agents) and is associated with certain comorbidities, such as T2DM. Compared to EOP, LOP patients seem to be influenced by external stressors and react with high levels of anxiety. On a microscopic level, LOP demonstrate a high infiltration of $CD4^+$ and $CD3^+$ cells in the epidermis of PP sections, whilst histological sections look in general more inflammatory.

6.5 Future aims

The data presented here suggest that EOP and LOP are clinically different and follow a different heritance pattern. These findings exclusively apply to Caucasian patients, who were recruited for the purposes of this thesis. Similar to this study, previous findings on the topic were produced from national-level studies. A multi-ethnic, multi-centre observational study is required to confirm the current observations in the same as well as other ethnic groups. A detailed questionnaire, similar to the one used in the prospective study, is required, that will include detailed information on age of onset and gender of patients and relatives. In addition, the observed associations of EOP and LOP with specific phenotypes warrant a large, observational cohort, including hospital and community based populations, where patients will be clinically evaluated for their clinical phenotype and response to different therapeutic agents.

The higher prevalence of comorbidities and autoimmune inflammatory diseases in patients with psoriasis is well established. Current research focuses to identify those relationships between other diseases and psoriasis and thus, improve the quality of patients' monitoring. The identified link between certain comorbidities and LOP, in this study, requires further research, with a large study sample and detailed information on diagnosis of these comorbidities, such as clinical and laboratory, detailed information on age of onset of the comorbidity, relation to psoriasis onset, family history of psoriasis and comorbidity, as well as disease severity and response to therapy. Previous studies have mostly used retrospective data or prospective surveys that were based on patient recall data. Moreover, it would be interesting to identify potential genetic links among associated co-morbidities and the two subtypes of psoriasis and this can be explored by

conducting a large, long-term biobank study of blood samples from EOP and LOP patients, as well as controls.

Previous research indicates that psychological disability plays a key role in response to treatment (Fortune et al., 2003). The current observations that LOP is a clinically more anxious group of patients needs further investigation. The psychosocial impact could be further explored with additional psychometric questionnaires, as well as explore whether anxiety levels link with therapeutic responsiveness in LOP vs EOP.

This thesis has also highlighted histological and immunological differences between EOP and LOP. More specifically, the current results presented a central role of $CD4^+$ T-cells in the epidermis of LOP, PP skin. It is therefore of great importance that future studies further explore the role of $CD4^+$ T-cells in the pathogenesis of LOP. As mentioned in previous chapters, it would be essential to identify the immune phenotype of these cells (by identifying cellular markers and produced cytokines), as well as the cellular and humoral microenvironment which induces their activation. In addition, it would be interesting to determine the adhesion molecules that enable these cells to enter in the epidermis and maybe help them sustain the chronic inflammation. A larger biopsy study is hence required which would confirm the current observations and explore additional markers. There is also increasing evidence that NKT and Tγδ cells play significant role in the immunopathogenesis of psoriasis, by communicating with other immune cells (dermal DCs,Th1 and Th17 cells) and stimulating the inflammatory process. It would be therefore important to investigate whether these cell-populations differ between EOP and LOP. Regarding the observed histological differences, it would be advantageous if the H&E findings could be compared against more sensitive imaging techniques, such as the confocal microscopy.

6.6 Potential Clinical Application

As described in the introduction of this thesis, it is possible that in the future what we once looked as psoriasis, will turn out to be various pathophysiologically distinct but phenotypically alike dermatoses, with different mechanisms and hence, different therapeutic management (Farley et al., 2009). Recent studies have also underlined the association of psoriasis with other chronic and debilitating conditions and the subsequent huge economic burden (Kim et al., 2010). Taken together, there is a clear need towards a proper classification of patients according to established criteria which will then lead to more effective treatment. This thesis demonstrated that the classification proposed by Henseler and Christophers in 1985 divides psoriasis patients in two distinct subphenotypes, with genetic and pathogenic diversity (Henseler and Christophers, 1985). The direct effect of this is that these patients might potentially require different therapeutic management. This is also of great importance as it might explain the high relapse rate seen in psoriasis patients, even after the use of very potent therapeutic agents.

Observed phenotypic differences	EOP	LOP
Clinical findings	• Severe, eruptive disease • Guttate, erythrodermic and pustular psoriasis • Small, thick plaque psoriasis • Frequent use of 3rd line treatments • Negative association with AIT	• Stable disease • Scalp psoriasis and PPP • Small and large-thin plaque psoriasis • Nail bed changes • Higher levels of anxiety • Positive association with T2DM • Frequent use of 1st line treatments and phototherapy
Histological findings	• Less prominent inflammatory component	• Prominent lymphocytic infiltration
Immunological findings	• $CD4^+/CD8^+ <1$	• Epidermal influx of $CD4^+$ cells • Higher count of $CD3^+$ cells • $CD4^+/CD8^+ \geq 1$

Table 6.1 Summary of findings of this thesis.

This table presents a summary of the results presented in this thesis and identified clinical, histological and immunological differences between early onset-EOP and late onset psoriasis-LOP. AIT= autoimmune thyroiditis, PPP= palmoplantar pustulosis, T2DM=type 2 Diabetes Mellitus

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Appendix A

Appendix A includes the ethics approved protocol and study designed questionnaire of the prospective cohort, as well as the psoriasis proforma of the retrospective cohort, used in the clinical study (**Chapter 3**). These elements are included as reprints (windows enhanced metafiles and scanned copies). Finally, additional photos of EOP and LOP participants have been placed here.

1. Ethics approved protocol for the questionnaire study

MANCHESTER 1824

The University of Manchester

Salford Royal NHS Foundation Trust

University Teaching Hospital

PROTOCOL

STUDY TITLE: **Phenotypes of Psoriasis**

Secondary title: **Exploring the phenotypic heterogeneity in patients with psoriasis**

Sponsor:
The University of Manchester

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Aim

The main objective of this study is to identify the clinical, demographic and psychosocial differences between early onset (type I) and late onset (type II) chronic plaque psoriasis. This will further help us to better distinguish between the two groups and aid us in better treating them in a more effective manner.

We will also look at other phenotypic variants of psoriasis, focussing on the clinical, demographic and psychosocial differences that may exist between these different types of psoriasis.

In order to achieve these objectives, we will perform a questionnaire and full examination in patients with psoriasis.

Background

Exploring the basis of phenotypic heterogeneity in psoriasis

Psoriasis is a chronic, non-contagious, recurrent, inflammatory skin disease, with an incidence of 2% among populations of European origin. Psoriasis appears to be both genotypically and phenotypically heterogenous. Psoriasis vulgaris (chronic plaque psoriasis) is the commonest clinical type of psoriasis in adults, characterized by the presence of well-demarcated, salmon-coloured plaques covered with silver-white scaling. Histology reveals marked epidermal hyperplasia, dilated and tortuous blood vessels in the dermis and a dermal inflammatory infiltrate of prominently lymphocytes.

Despite psoriasis being a highly visible disorder, no specific diagnostic test exists either to confirm the clinical diagnosis or predict future appearance of the disease. From the pool of diagnostic strategies that exist in clinical practice, the most common used in dermatology to diagnose psoriasis has been pattern recognition. Therefore, most of the classification schemes that exist for psoriasis are based on the morphology of psoriatic lesions and pattern recognition.

An identification of two psoriatic subtypes based on the “age of disease onset” has been explored by Henseler and Christophers, in 1985. Two distinct disease types were identified:
1) Presentation of early onset or type I psoriasis is featured by a younger age of onset (< 40 years), a higher incidence of guttate and eruptive type of psoriasis, more frequent exacerbations and often relapses usually following β-hemolytic streptococci infections, a higher incidence of Koebnerization, an increased psychological impact, while nail and facial involvement are commonly seen. Type I psoriasis patients are also more likely to have a first degree relative and a more aggressive clinical course of the disease.
2) Late onset or type II psoriasis includes a milder disease and a more stable clinical course of plaque-type psoriasis and a weak association of positive family history. Henseler and Christophers observed that HLA-Cw*0602 positive patients experienced an earlier disease onset and therefore discussed about a different genotype and mode of

inheritance between these two types of psoriasis. The above pattern recognition and classification of psoriasis has been explored in a number of studies and has been widely accepted by the Psoriasis research community, but there is little supporting literature specifying the clinical (symmetrical lesions with an active edge and a clear centre, thickness of the psoriatic plaques) pattern of the disease, the underlying pathogenesis and response to treatment. In addition there are no follow up studies correlating disease outcome between late onset psoriasis and early onset psoriasis patients. Furthermore, there are no data correlating late onset psoriasis with late onset psoriatic arthritis.

Moreover, recent observational studies have demonstrated that there is a strong relationship between psoriasis and co morbidities such as metabolic syndrome and depression. It is not clear though whether there these comorbidities exist in both type I and type II chronic plaque psoriasis.

As mentioned previously, psoriasis is a non-uniform disease, with a number of phenotypic variations and an increasing health, social and economic impact. Therefore, additional evidence-based research addressing a sufficient classification system is needed to elucidate the puzzling picture of psoriasis. By better demarcating psoriatic phenotypes according to clinical, demographic and psychosocial features, we may also recognize new cost-effective and individualized treatment strategies.

Study design

We propose to perform a clinical study incorporating some basic questionnaires. In order to achieve that, we shall recruit 500 volunteers with chronic plaque psoriasis, aged 18 years old and over, who will be recruited and divided into two groups, 250 with type I (early onset) chronic plaque psoriasis (age of disease onset 18-40 years old) and 250 with type II chronic plaque psoriasis (age of disease onset>50 years old). We will then try to identify the phenotypic differences (demographic, clinical and psychosocial) that might exist between the above groups.
We will also recruit those patients with age of disease onset between 40 and 50 years old (estimated 100 patients) but believe they may be heterogeneous and as such will require careful analysis. We will also perform the same process with patients diagnosed with other psoriasis phenotypes (e.g pustular – estimated 100 patients) and compare the collected data with chronic plaque psoriasis patients.

For the study purpose, we will have designed a Psoriasis Clinical Assessment form (the study questionnaire), which will be given to our research participants (psoriatic patients), to help us assess the status of their disease. The above questionnaire is divided into two parts:

Psoriasis clinical assessment form design

A) The first part will be completed by the clinician. The information which will be recorded in this section is: demographic characteristics, disease severity, disease duration, concomitant medication use, past medical history, family health history, social history

(patients' profile), risk factors and associated comorbidities. The time required for this section is approximately 30 minutes. (Please see attached proforma at the end of the protocol)

B) The second part will be completed by the patient and includes 4 simple, self-report questionnaires aiming to evaluate the psychosocial impact of the disease. These are the Dermatology Life Quality Index (DLQI), the Hospital Anxiety and Depression scale (HADS), the Penn State Worry Questionnaire (PSWQ) and the Beck Depression Inventory (BDI-II). The time required to complete this section is approximately 30 minutes. (Please see attached proforma at the end of the protocol)

This study requires a one hour visit to the Dermatology Unit, Salford Royal NHS Foundation trust, ideally at the time of the patient's clinic appointment.

Participants

Recruitment

Potential research participants will be identified by our database of psoriatic patients from our weekly Psoriasis clinic, at Salford Royal NHS Foundation Trust. Furthermore, we shall post advertisements, about the study at the K1 Dermatology/Rheumatology Ward, at Salford Royal NHS Foundation Trust. In addition, the fully trained study doctor will be handing out information sheets to potential participants attending our local psoriasis clinics. All participants will be screened to ensure they fulfil the inclusion criteria (and none of the exclusion criteria). Volunteers who contact the team (usually by telephone or electronic mail) will be provided with initial information about the study. If they are eligible they will be sent full information. The potential participant will have as much time as he/she needs to decide whether to take part in the study with a minimum period of 24 hours. At an agreed time, the volunteer will be contacted or will contact the team themselves with their decision. Those who express a willingness to take part will be invited to attend the Dermatological Science Unit at Salford Royal Hospital NHS Foundation Trust. At this visit, participation will be discussed fully and those who remain willing to take part will be recruited to the study. All participants will be asked to read and sign a consent form. A letter will notify each participant's family practitioner that his or her patient will be taking part in the study. The majority of patients will already attend Salford Royal NHS Foundation trust for their routine care, these individuals will ideally be seen around there clinic appointment time. For those responding to the external adverts a time convenient for them to attend the Dermatopharmacology Unit will be made.

Inclusion / exclusion criteria

Inclusion	Exclusion
Patients with a confirmed diagnosis of any type of psoriasis by a dermatologist, male or female;18 years old and over;	Patients unable to provide a written informed consent;

Study procedures

Materials and methods

An information leaflet will be sent to prospective participants. They will be given at least 24 hours to decide whether or not to take part.

Visit 1-Day 1:

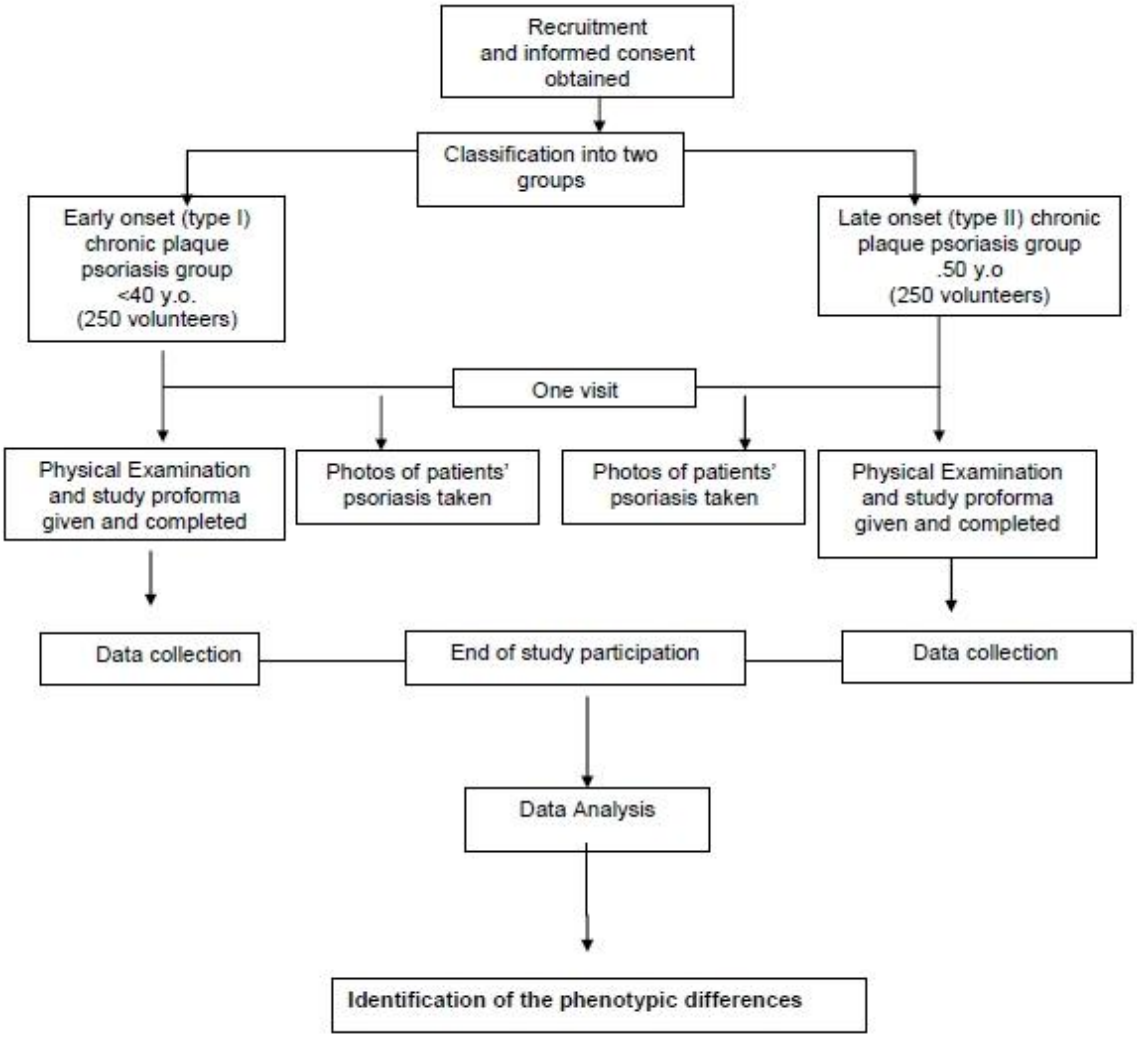
1. Witnessed, written informed consent;
2. Interview and clinical examination, conducted by the fully trained study doctor;
3. Complete the study questionnaire;
4. Digital photos of the participants' psoriatic rash taken and safely stored in the encrypted NHS Trust approved medical photography site of Salford Royal NHS Foundation Trust

During their visit at the hospital, on day 1, research participants will be seated in a quiet room. Written informed consent will be taken which will be followed by a medical history and skin examination including a Psoriasis Area Severity Index (PASI) assessment and the completion of part A of the study questionnaire. The first section of the study questionnaire will be completed by the fully trained and qualified study doctor, at Salford Royal NHS Foundation Trust. Thereafter, participants will be asked to complete part B of the psoriasis clinical assessment form. This part of the study questionnaire will be completed, whilst the patient is seated in a quiet room, by the study doctor, at Salford Royal NHS Foundation Trust.
Finally, photos of the different patterns of volunteers' psoriasis will be taken, by a fully qualified and trained member of our research group.

Study outline

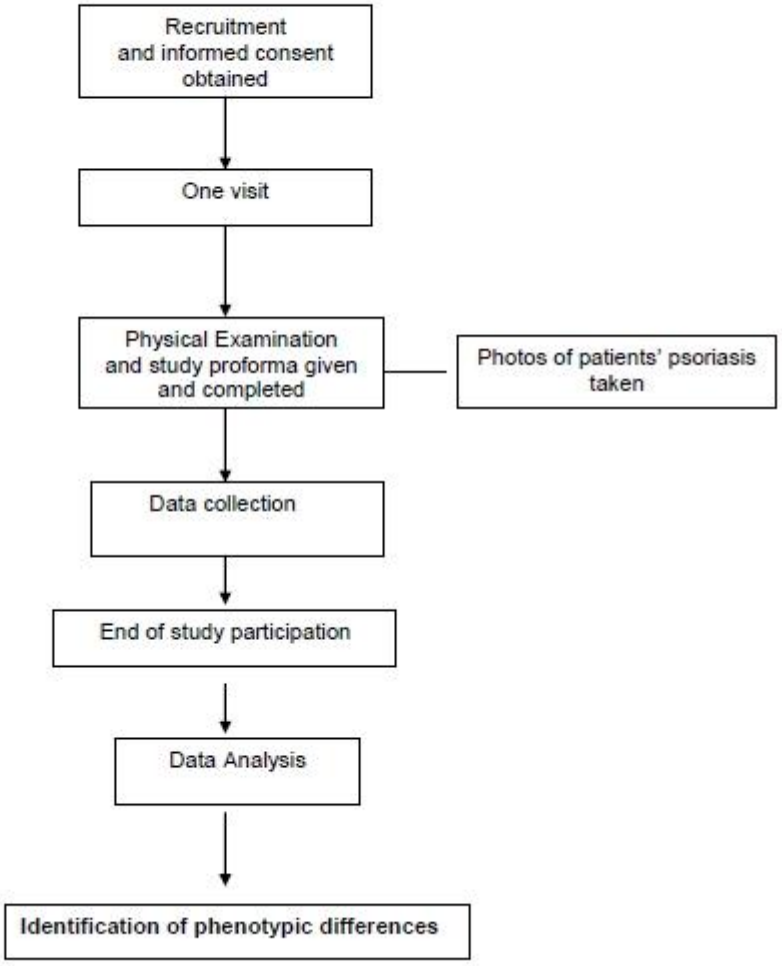
COMPARISON OF PSORIATIC PHENOTYPES

Type I versus type II chronic plaque psoriasis



COMPARISON OF PSORIATIC PHENOTYPES (CON'T)

Sebopsoriasis / Guttate/Generalized/Loclaized pustular psoriasis/ other phenotypes



2. Study questionnaire of the prospective cohort

MANCHESTER 1824

The University of Manchester

DERMATOLOGY DIRECTORATE

Salford Royal Hospital NHS Trust

PSORIASIS CLINICAL ASSESSMENT

Place sticker here

(Drawing of the patient's family pedigree chart)

Section A: To be completed by the physician

Name:

Gender : M / F

Date of Birth: d d m m y y y y

Hospital No. :

Address

Tel. :

GP Name/Address:

Date of Assessment: d d m m y y y y

1. Age of disease onset (yrs) : .

2. Duration of Disease (yrs) :

3. **Family Health Tree** :

Drawing up guidelines:

Normal male family member

Normal female family member

Family members with psoriasis

Drawing-up guidelines for co morbidities:

i.e Normal male family member with…

Psoriatic arthritis

Depression

Other autoimmune diseases (DM,Crohn's, thyroiditis, coeliac disease,other)

Please **draw** the patient's pedigree chart and **indicate both** patient and affected family members' age of disease onset, at the next page. Please **specify** the autoimmune disease, if any, on the pedigree chart:

Social factors

4. Alcohol (units /week):

1 unit of alcohol= i.e. a **half pint** (284 ml) **of beer** of 3.5% abv, a **small glass** (125 ml) of 8% abv **wine,** a **small glass** (50 ml) of **sherry, fortified wine**, or **cream liqueur** (approx. 20% abv), a **single pub measure** (about 25 ml) of distilled **spirits**

5. Tobacco (cigarettes per day) :

6. Occupation:

7. Marital Status:

Treatment history

5. **Current** treatments for psoriasis:

1. Topical agents	3. Systemic Treatment
2. Light (UV) treatment	4. Biologic agents

6. **Past Systemic** Treatments

Name:
Date:
Efficacy (circle) Excellent Good Poor
Side Effects:

Name:
Date:
Efficacy (circle) Excellent Good Poor
Side Effects:

Name:
Date:
Efficacy (circle) Excellent Good Poor
Side Effects:

Past Topical Treatments

Name:
Date:
Efficacy (circle): Excellent Good Poor
Side Effects:

Name:
Date:
Efficacy (circle): Excellent Good Poor
Side Effects:

Name:
Date:
Efficacy (circle): Excellent Good Poor
Side Effects:

Name:
Date:
Efficacy (circle): Excellent Good Poor
Side Effects:

Past UV therapy:

Broadband UVB:
Narrowband UVB:
Total body PUVA:
Hand and foot PUVA:
No of treatments:
Efficacy (circle): Excellent Good Poor
Side effects:

7. List of medications *(other than for psoriasis)*

1.	9.
2.	10.
3.	11.
4.	12.
5.	13.
6.	14.
7.	15.
8.	16.

Number of hospital admissions for psoriasis:

0 1 2 3 4 (circle)

Physical examination

8. Blood Pressure (mmHg) :
9. Weight (Kg) :
10. Height (cm):
11. Waist circumference (cm):
12. BMI:

13. Are there any factors that **exacerbate** the patient's psoriasis? If so, please specify.

Medications:
Sunlight:
Stress:
Other Factors:

14. Do **Sore Throats / Tonsillitis exacerbate** the patient's psoriasis?

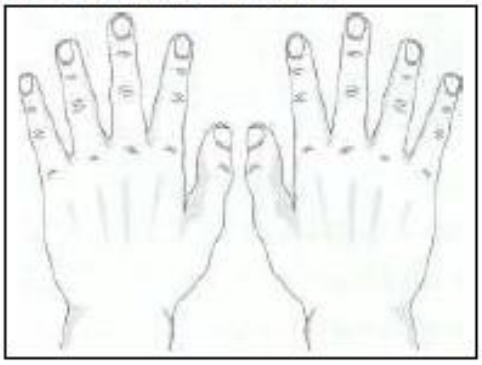
YES NO

15. Are there any factors that **improve** the patient's psoriasis? If so, please specify.

Medications :	
Sunlight:	
Other Factors:	

16. Finger nails involved? YES NO

If YES, which nails *(colour nails)*



Toe nails involved? YES NO

If YES, how many?

Please circle any nail change that apply to the patient

Nail plate changes	Pits Leukonychia Lunular red spots
Nail bed changes	Onycholysis Hyperkeratosis Oil drop (salmon patches)disocoloration Splinter nail bed

17. <u>Psoriatic Arthritis (PsA)</u>

17a. Has the patient been diagnosed with PsA? YES NO

17b.If yes, was the PsA diagnosis made by a Rheumatologist? YES NO

18. Current treatment for PsA?

1.
2.
3.
4.
5.

19 a. Has the patient ever experienced any **psoriasis-free interval(s)**? YES NO

If so, please indicate "when" and "how long" this remission has lasted and list any factor(s) associated with the disease remission? i.e. current /previous treatments, sunny holidays, lifestyle change, smoking/alcohol cessation, other:

Additional question addressed to female patients

19 b. Has the patient noticed any flares or remissions in their psoriasis related to **pre-menstrual stage of their cycle?** YES NO

If so, please specify if it was flare or remission:

19 c. Has the patient noticed any flares or remissions in their psoriasis related to **pregnancy and/or post-partum**? YES NO

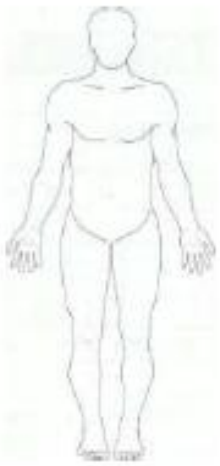
If so, please specify if it was flare or remission

19d. Has the patient noticed any flares or remissions in their psoriasis related to **menopause**? YES NO

If so, please specify if it was flare or remission

20. Psoriasis Distribution - *shade on mannequin*

Front **Body Surface Area Involved** **Back**



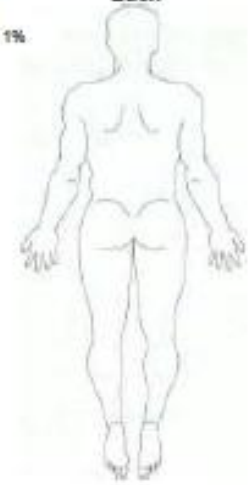
Surface Area Estimation: One Handprint = 1%

HEAD

UPPER LIMBS

TRUNK

LOWER LIMBS



21. What type of psoriasis does the patient currently have?
What is/was the clinical type of the affected family member(s), if any?

Clinical type of psoriasis	Patient (currently)	Affected family member(s)
Stable psoriasis		
Unstable		
Chronic plaque psoriasis • Small (<3cm diam) • Mix of both • Large(>3cm diam) • Thin (0-1 NPF score) • Intermediate (2 NPF score) • Thick (3-5 NPF score)		
Seborrhoeic psoriasis		
Flexural/Intertriginous		
Scalp		
Palms/soles (non pustular)		
Erythrodermic		
Guttate psoriasis		
Generalised pustular psoriasis		
Localised pustular psoriasis • Acrodermatisis Continua		
• Palmoplantar pustulosis		
Other Please specify:		

22. Patient Global Psoriasis Assessment

Considering all the ways your psoriasis affects you, please grade from 0 (very good-no symptoms) to 10 (very poor-severe symptoms) your condition, to show how you are feeling today

23. Has the **clinical pattern** of the patient's psoriasis changed since first diagnosed? YES NO
If so, please specify this change and list any aggravating factor(s) that triggered this change? i.e. previous treatments such as Cyclosporine, MTX, PUVA, UVB, other:

Date : d m y

PSORIASIS AREA & SEVERITY INDEX (PASI)

Score for Symptom and Area for head,trunk, upper- and lower limbs

Score	0	1	2	3	4	5	6
Erythema Infiltration Desquamation	None	Slight	Moderate	Severe	Very Severe		
Area %	**0**	**<10**	**10<30**	**30<50**	**50<70**	**70<90**	**90-100**

Please Score :	HEAD	UPPER LIMBS	TRUNK	LOWER LIMBS
Erythema (E)				
Infiltration (I)				
Desquamation (D)				
Sum = E + I + D				
Area				
Sum x Area =				
	x 0.1 =	x 0.2 =	x 0.3 =	x 0.4 =
TOTAL (PASI)				

Section B-TO BE COMPLETED BY THE PATIENT

Please tick √ the appropriate boxes

Patient's racial and ethnic background	Patient's ethnic/racial group	Family Members' ethnic/racial group			
		Father	Mother	Grandfather	Grandmother
White					
White British					
Irish					
Any Other White background (If other, please print ethnicity in the appropriate check boxes)	print ethnicity	print ethnicity	print ethnicity	print ethnicity	print ethnicity
Black					
Caribbean					
African					
Other Black groups	print ethnicity	print ethnicity	print ethnicity	print ethnicity	print ethnicity
Asian					
Indian					
Pakistani					
Bangladeshi					
Any other Asian background	print ethnicity	print ethnicity	print ethnicity	print ethnicity	print ethnicity
Chinese					
Han Chinese					
Other Chinese background	print ethnicity	print ethnicity	print ethnicity	print ethnicity	print ethnicity
Mixed (Patients may have chosen to provide two or more races by **checking two or more** race response check boxes)					
Some Other Racial / Ethnic group	print ethnicity	print ethnicity	print ethnicity	print ethnicity	print ethnicity

White. A person having origins in any of the original people of Europe, the Middle East, or North Africa, including report entries such as Irish, German, Italian, Lebanese, Near Easterner, Arab, or Polish.
Black A person having origins in any of the Black racial groups of Africa. It includes people who indicate their race as "Black, African, Caribbean" or "other Black groups"
Asian. A person having origins in any of the original people of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, India, Japan, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.
Chinese.A person having origins in any of the original the original people of China, including "Han Chinese", and "other nationalities" (such as *Zhuang, Uygur, Hui, Yi, Tibetan, Miao, Manchu, Mongol, Buyi, Korean,etc*)
Some other race. Includes all other responses not included in the "White", "Black ","Asian" and "Chinese" ethnicity categories described above. Respondents with report entries such as multiracial, mixed, interracial are included here.

TO BE COMPLETED BY THE PATIENT

Past Medical History

Health history : Please complete the following illnesses/injuries checklist below

Have you ever had...?	YES	NO	YEAR of Diagnosis	Do you relate it to you skin condition ... (i.e. as the triggering or exacerbating factor) YES NO Comments
Physical Trauma 1.Traffic accident, head injury, skull fracture, whiplash				
2.Hospitalizations for illness or injury				
3.Fractures of any type				
4.Ulcers ,scars or identifying marks				
5.Back injury, herniated disc				
Metabolic syndrome 6.Diabetes				
Type I				
Type II				
7.Increased BMI (BMI>30,indicating obesity)				
8.Non-alcoholic fatty liver				
9.Abnormal liver function tests				
10.Elevated triglycerides				
11.Low HDL				
12.Increased levels of uric acid				
Cardiovascular disease 13.High blood pressure				
14.Angina				
15.Arrhythmia				
16.Myocardial Infarction				
17.Peripheral vascular disease				
18.Cerebrovascular accident (stroke)				

Have you ever had...?	YES	NO	YEAR	Do you relate it to you skin condition ...? (i.e. as the triggering or exacerbating factor) YES NO Comments
Inflammatory diseases 19.Inflammatory Bowel Disease i.e. Crohn's disease or ulcerative colitis				
20.Thyroid disease				
21.Multiple Sclerosis				
Respiratory /Pneumonal 22.Tuberculosis				
23. Obstructive sleep apnea				
24.Asthma				
Renal involvement 25.Raised Creatinine				
26.Renal disease				
Other 27.Tumor or cancer				
28.Treatment for Anxiety / Depression				

Please complete the above health history

TO BE COMPLETED BY THE PATIENT

- If YES in question 27,please specify the type of cancer , body location and date of diagnosis :

_____

- If YES in question 28, please specify if you ever had any psychologically traumatic event (i.e. family death, divorce or separation, moving house, changing job, redundancy and treatment for anxiety or depression):

_____

- Do you have any allergies to foods? To medications? To other substances? YES NO
  If so, please specify the type(s) of allergy, you have.

_____

If you have a drug allergy, please list all the culprit medications:

1.
2.
3.
4.
5.

- Do you have any other health concerns not mentioned above?

_____

PSYCHOSOCIAL FACTORS

TO BE COMPLETED BY THE PATIENT

DERMATOLOGY LIFE QUALITY INDEX

DLQI

Hospital No: Date: Score:

Name: Diagnosis:

Address:

The aim of this questionnaire is to measure how much your skin problem has affected your life OVER THE LAST WEEK. Please tick one box for each question.

1.	Over the last week, how itchy, sore, painful or stinging has your skin been?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	
2.	Over the last week, how embarrassed or self conscious have you been because of your skin?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	
3.	Over the last week, how much has your skin interfered with you going shopping or looking after your home or garden?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	Not relevant ☐
4.	Over the last week, how much has your skin influenced the clothes you wear?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	Not relevant ☐
5.	Over the last week, how much has your skin affected any social or leisure activities?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	Not relevant ☐
6.	Over the last week, how much has your skin made it difficult for you to do any sport?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	Not relevant ☐
7.	Over the last week, has your skin prevented you from working or studying?	yes no	☐ ☐	Not relevant ☐
	If "No", over the last week how much has your skin been a problem at work or studying?	A lot A little Not at all	☐ ☐ ☐	
8.	Over the last week, how much has your skin created problems with your partner or any of your close friends or relatives?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	Not relevant ☐
9.	Over the last week, how much has your skin caused any sexual difficulties?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	Not relevant ☐
10.	Over the last week, how much of a problem has the treatment for your skin been, for example by making your home messy, or by taking up time?	Very much A lot A little Not at all	☐ ☐ ☐ ☐	Not relevant ☐

Please check you have answered EVERY question. Thank you.

TO BE COMPLETED BY THE PATIENT

Hospital Anxiety and Depression Scale

You are asked to choose one response from the four given for each interview. You should give an immediate response and be dissuaded from thinking too long about your answers. The questions relating to anxiety are marked "A", and to depression "D". The score for each answer is given in the right column.

	Yes definitely	Yes sometimes	No, not much	No, not at all
1. I wake early and then sleep badly for the rest of the night.	3	2	1	0
2. I get very frightened or have panic feelings for apparently no reason at all.	3	2	1	0
3. I feel miserable and sad.	3	2	1	0
4. I feel anxious when I go out of the house on my own.	3	2	1	0
5. I have lost interest in things.	3	2	1	0
6. I get palpitations, or sensations of 'butterflies' in my stomach or chest.	3	2	1	0
7. I have a good appetite.	0	1	2	3
8. I feel scared or frightened.	3	2	1	0
9. I feel life is not worth living.	3	2	1	0
10. I still enjoy the things I used to.	0	1	2	3
11. I am restless and can't keep still.	3	2	1	0
12. I am more irritable than usual.	3	2	1	0
13. I feel as if I have slowed down.	3	2	1	0
14. Worrying thoughts constantly go through my mind.	3	2	1	0

Anxiety questions : 2, 4, 6, 8, 11, 12, 14

Depression questions : 1, 3, 5, 7, 9, 10, 13

If you have scored 8 or higher, in the questions related to depression (D), please complete the Beck Depression Inventory questionnaire.

TO BE COMPLETED BY THE PATIENT

The Penn State Worry Questionnaire

Please read each statement and rate each of the following statements on a scale of 1 ("not at all typical of me") .There are no right or wrong answers. Do not spend too much on any one statement. This assessment is not intended to be a diagnosis. If you are concerned about your results in any way, please speak with a qualified health care professional.

1= Not at all typical of me

2= Rarely typical of me

3= Somewhat typical of me

4= Often typical of me

5= Very typical of me

	Not at all typical of me				Very typical of me
1. If I do not have enough time to do everything, I do not worry about it.	1	2	3	4	5
2. My worries overwhelm me.	1	2	3	4	5
3. I do not tend to worry about things.	1	2	3	4	5
4. Many situations make me worry.	1	2	3	4	5
5. I know I should not worry about things, but I just cannot help it.	1	2	3	4	5
6. When I am under pressure I worry a lot.	1	2	3	4	5
7. I am always worrying about something.	1	2	3	4	5
8. I find it easy to dismiss worrisome thoughts.	1	2	3	4	5
9. As soon as I finish one task, I start to worry about everything else I have to do.	1	2	3	4	5
10. I never worry about anything.	1	2	3	4	5
11. When there is nothing more I can do about a concern, I do not worry about it any more.	1	2	3	4	5
12. I have been a worrier all my life.	1	2	3	4	5
13. I notice that I have been worrying about things.	1	2	3	4	5
14. Once I start worrying, I cannot stop.	1	2	3	4	5
15. I worry all the time.	1	2	3	4	5
16. I worry about projects until they are all done.	1	2	3	4	5

Beck Depression Inventory questionnaire

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness

0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism

0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure

0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure

0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings

0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike

0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness

0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying

0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

3. Psoriasis clinic proforma of the retrospective cohort

The Salford psoriasis assessment proforma

DERMATOLOGY DIRECTORATE

Salford Royal Hospitals NHS
NHS Trust

PSORIASIS CLINICAL ASSESSMENT

Place sticker here

Name : .....

Address : .....

.....

Post Code : .....

Date of Birth : .....

Hospital No. : .....

Sex : M / F

Tel. : .....

G.P. : .....

.....

Date of Assessment : ..... / ..... / .....

1. Age of Onset (yrs) : .....
2. Duration of Disease (yrs) : .....
3. Family History :

○ Female ☐ Male
● Proband ■ Proband

4. Current Severity

(0 = mild, 10 = severe) : .....

5. What Exacerbates Psoriasis ?

Medications : .....

Other Factors : .....

6. Do Sore Throats / Tonsillitis Exacerbate Psoriasis ?

YES ☐ NO ☐

7. What Improves Psoriasis ?

Medications : .....

Other Factors : .....

8. Current Treatments :

.....

.....

.....

9. Past Systemic Treatments

Name : .....
Date : .....
Efficacy : .....
Side Effects : .....

Name : .....
Date : .....
Efficacy : .....
Side Effects : .....

Name : .....
Date : .....
Efficacy : .....
Side Effects : .....

10a. Past Topical Treatments

Name : .....
Date : .....
Efficacy : .....
Side Effects : .....

Name : .....
Date : .....
Efficacy : .....
Side Effects : .....

Name : .....
Date : .....
Efficacy : .....
Side Effects : .....

Name : .....
Date : .....
Efficacy : .....
Side Effects : .....

10b. Number of hospital admissions for psoriasis .....

11. Past Medical / Surgical History :

Diabetes	YES ☐	NO ☐
Hypertension	YES ☐	NO ☐
Cardiac Disease	YES ☐	NO ☐
Asthma	YES ☐	NO ☐
Hay Fever	YES ☐	NO ☐
Other	YES ☐	NO ☐

If other please state .....

12. Medication taken *(other than for psoriasis)*

.....
.....
.....

13. Allergies

Allergy .....
Type .....

Allergy .....
Type .....

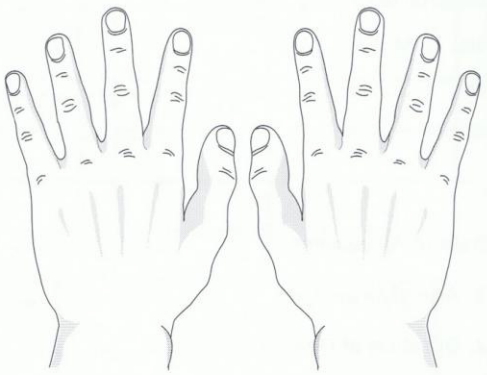
SOCIAL FACTORS

14. Alcohol (units/week) .....
15. Tobacco (per day) .....
16. Occupation .....
17. Marital Status .....

PHYSICAL

18. Blood Pressure (mmHg) .....
19. Weight (Kg) .....
20. Height (cm) .....
21. Finger nails involved ? YES ☐ NO ☐

If YES, which nails *(colour nails)*



22. Toe nails involved ? YES ☐ NO ☐

If YES, how many ?

.....

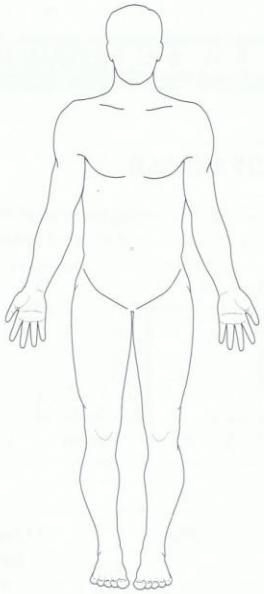
23. Psoriatic Arthritis ? YES ☐ NO ☐

If YES, which joints ?

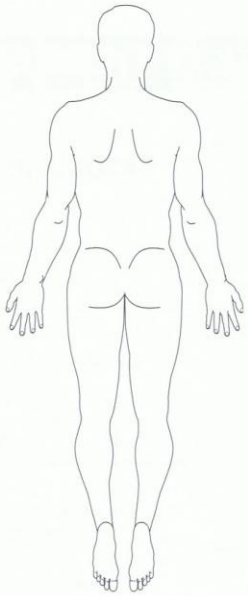
.....
.....

24. Psoriasis Distribution - *shade on mannequin*

FRONT



BACK



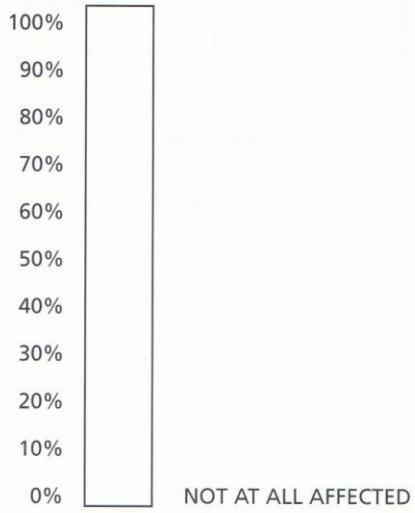
25. Type of Psoriasis

.....

.....

26. Visual Analogue Scale

Please indicate by placing an "X" on the scale below, the extent to which your psoriasis affects you in your day to day life at present.

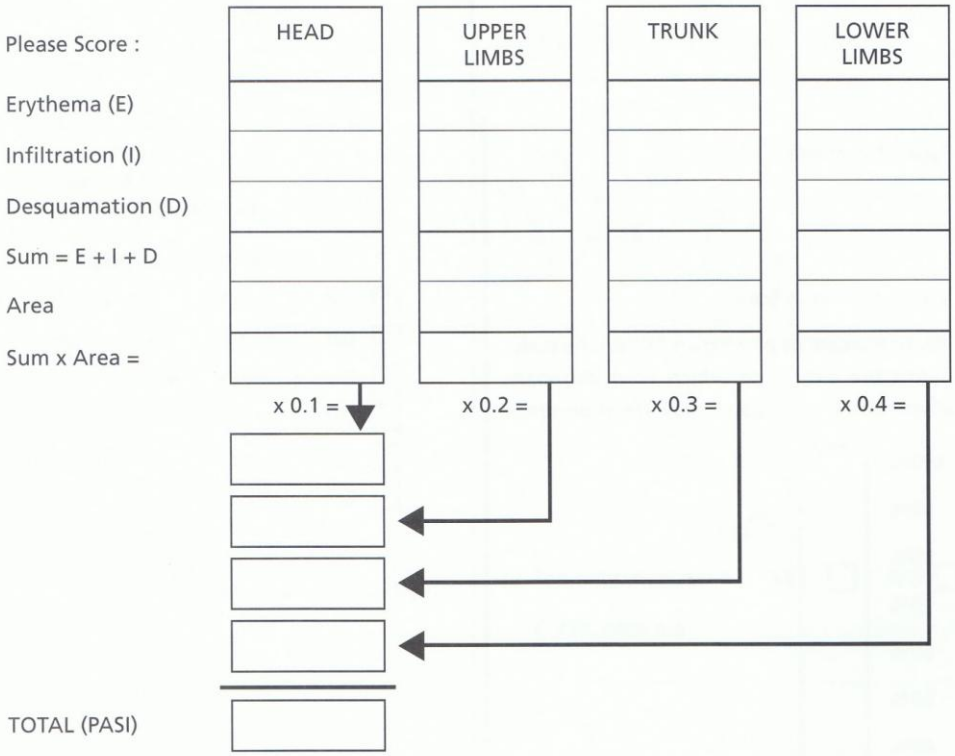


Date : d m y

PSORIASIS AREA & SEVERITY INDEX (PASI)

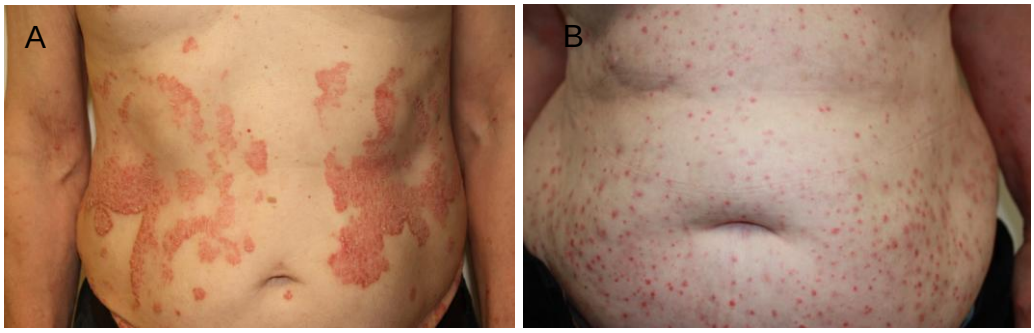
Score for Symptom and Area for head,trunk, upper- and lower limbs

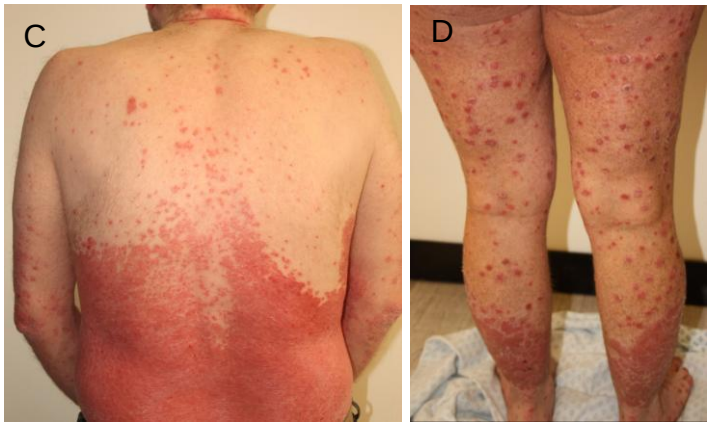
Score	0	1	2	3	4	5	6
Erythema Infiltration Desquamation	None	Slight	Moderate	Severe	Very Severe		
Area %	**0**	**<10**	**10<30**	**30<50**	**50<70**	**70<90**	**90-100**



4. Additional photos of participants from the clinical study

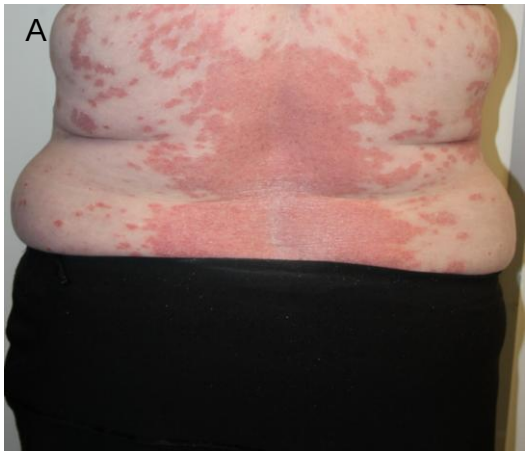
Early onset psoriasis-photos are from patients of the clinical study and consent was obtained.

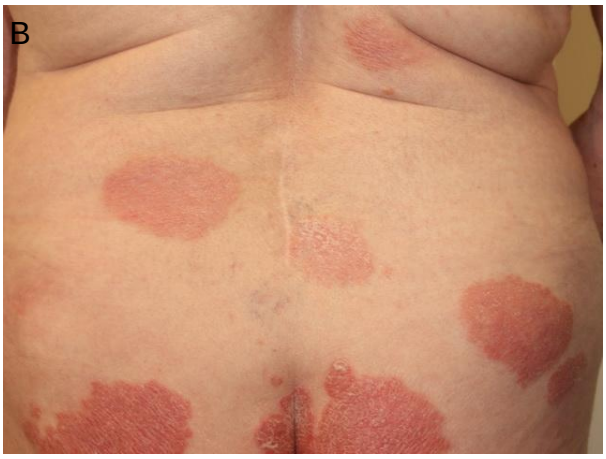


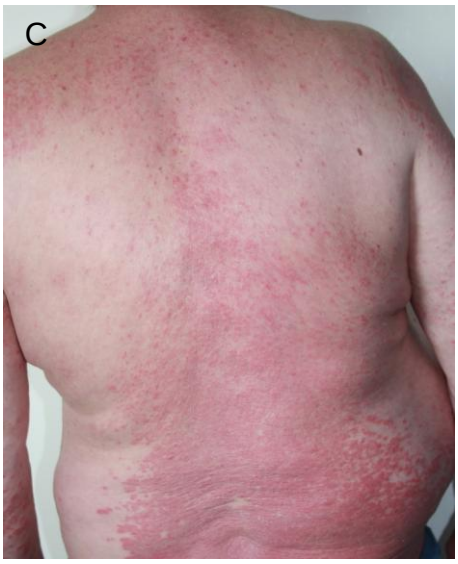


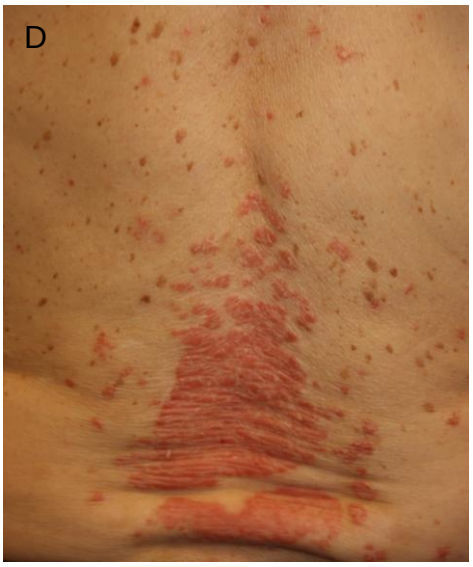
Early onset psoriasis-photos are from patients of the clinical study and consent was obtained.

Late onset psoriasis-photos are from patients of the clinical study and consent was obtained.









Appendix B

Appendix B includes a reprint of the ethics approved protocol of the biopsy study (**Chapters 4 and 5**), as well as a copy of the histological assessment form used for the histological evaluation of EOP and LOP (**Chapter 4**). All elements included here are either scanned copies or windows enhanced metafiles.

1. Ethics approved protocol for the "Immunogenetics of psoriasis"

PROTOCOL

An Investigation into the immunogenetics of psoriasis.

Dr Richard Warren
Professor Chris Griffiths
Dr Rachel Watson

Dermatopharmacology Unit
The University of Manchester
Irving Building
Salford Royal NHS Foundation Trust
Manchester
M8 9HT

Recruitment of patients with psoriasis

1. Hypothesis

There are genes / immunological factors that predispose to the development of psoriasis.

2. Aim

To invite patients with psoriasis to donate a blood sample from which DNA/ RNA and serum can be extracted in order to test candidate psoriasis susceptibility genes. In addition, a sub-group of these patients will be invited to provide skin biopsy and skin swab samples to correlate immunological and genetic factors which may be important in the pathogenesis of psoriasis.

3. Subjects

i. Recruitment

Individuals with psoriasis will be identified from hospital records by the staff within a Trust taking part in the study or in the psoriasis clinic at Salford Royal NHS Foundation Trust.

ii. Inclusion criteria

Patients and healthy volunteers must be $\geq$18 years of age.

The individual must be willing to participate in the study and satisfy the criteria for the presence of psoriasis diagnosed by a dermatologist (healthy volunteers must not have a diagnosis of psoriasis).

Patients must be of European Caucasian descent. It is necessary to restrict recruitment to those of European descent in order to reduce heterogeneity in the patient group for genetic studies. Frequencies of gene variants at different genetic markers differ between individuals from different ethnic groups and may introduce bias into any analysis.

iii. Exclusion criteria

Either the individual or consultant feels it is inappropriate for any reason for the patients to be enrolled.

4.

i Methods for genetic studies

1. Cases with psoriasis will be identified from a search of letters using the search term psoriasis or directly from the tertiary referral psoriasis clinic at Salford Royal NHS Foundation Trust. Healthy volunteers will be recruited via advertisement and from our existing database.
2. Trust staff will review the letters and case notes, if appropriate, to confirm the diagnosis.

Patients will be contacted by letter from their consultants or given letters directly in the tertiary referral psoriasis clinic at Salford Royal NHS

1. Foundation Trust. The letter will give a brief introduction to the proposed study and a patient information sheet would be included.
2. Patients will be asked to indicate whether they would like to take part in the study. This slip will be returned to clinic and positive responses only will be forwarded to the Dermatopharmacology Unit at the University of Manchester.

As part of their monitoring whilst receiving their treatment, patients with Psoriasis often have regular blood tests. We will request that, on the next occasion when blood is to be taken, an extra sample be taken at the same time. A separate venepuncture will not be required and this will minimise any inconvenience to the patient.

3. Patients identified through the psoriasis clinic at Salford Royal NHS Foundation Trust will have the blood sample taken at there next clinic visit.
4. The phlebotomist will be asked to take an appropriate blood sample to allow DNA extraction, in addition to the patient's routine sample requirements.
5. Each adult that agrees to take part in the study will be required to provide 2 samples of 10mls of blood from which DNA is extracted for the genetic analysis. Serum will also be stored.
6. A clinical proforma will be completed for each blood sample that is received.

ii Methods for skin biopsy / swabs

1. A sub-group of patients identified as part of the genetics investigations will be asked to provide skin biopsy and skin swab samples. All of these patients will be specifically identified from the tertiary referral psoriasis clinic at Salford Royal NHS Foundation Trust.
2. Patients will be given a letter of invitation by their consultant in clinic explaining what these additional investigations will entail.
3. Patients expressing an interest will then be invited to the Dermatopharmacology unit, Salford Royal NHS Foundation Trust. Skin swabs will be taken from sites of active psoriasis.
4. Skin biopsies (up to 4) will be taken from lesional (up to 2) and non-lesional skin (up to 2).

1. Funding

All equipment and postage costs will be met by the Dermatopharmacology Unit, but no additional funding for the study is available. The study will be registered on the NIHR portfolio so support costs can be requested.

The Dermatopharmacology Unit, the University of Manchester, will provide clinical consumables. Funding for these is provided through a 5-year programme grant to the Unit.

2. Time commitment from PCT/Acute Trust staff

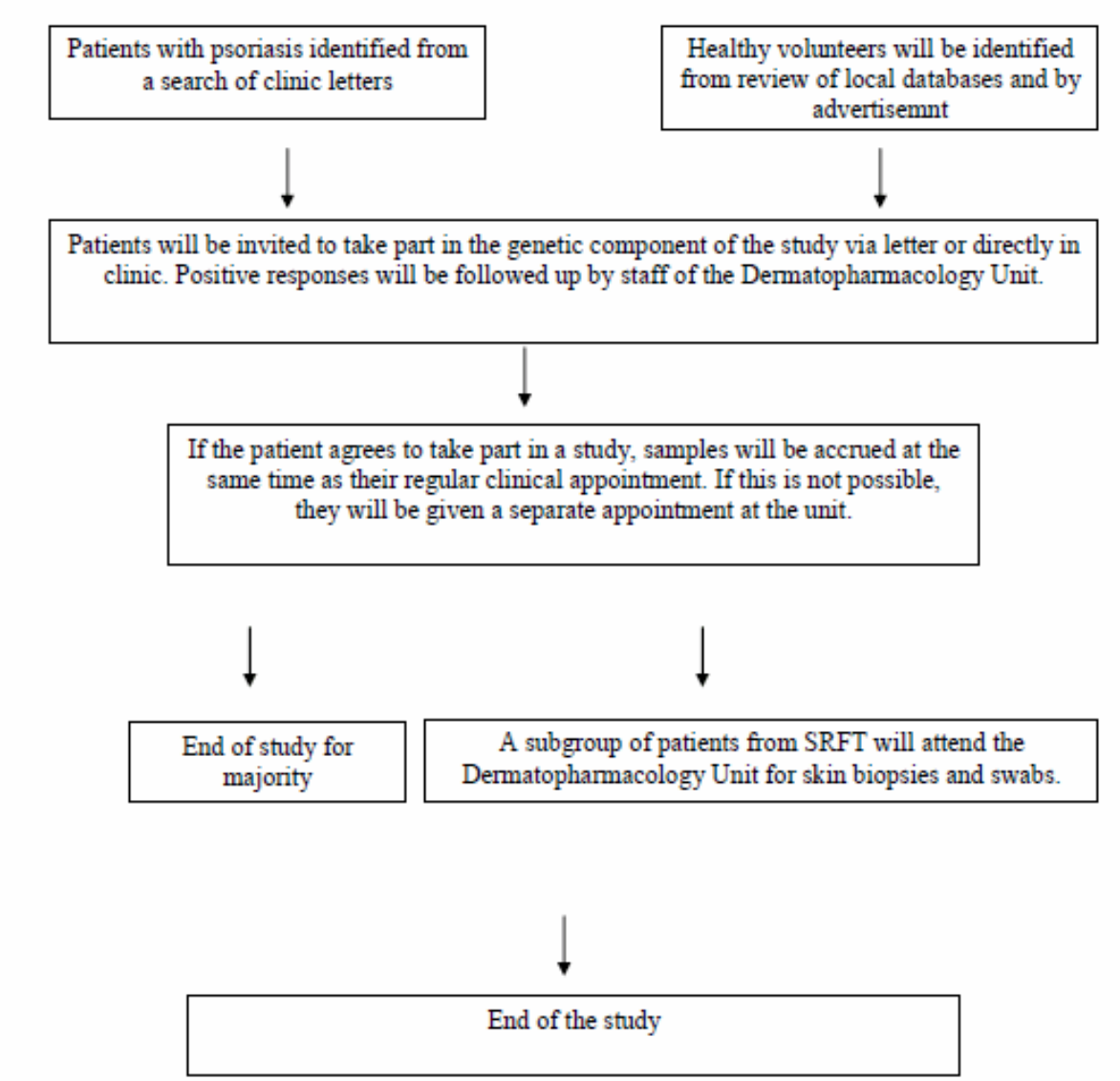
Constructing a list of patients and collating their positive/negative responses to forward to the University will be the responsibility of a secretary/nurse at site, due to data protection regulations; the proforma will need to be completed (1 page only) so a maximum of 10 minutes.

1. Other resource commitment by PCT/Acute Trust

Nil

2. Sponsor of study

The University of Manchester



Version 1, dated 04/05/10

2. Study-specific psoriasis histological assessment score

Phenotypes of Psoriasis Study		
Slide ID=		
H&E GRADING SYSTEM FOR PSORIASIFORM DERMATITIS		
Morphological Features of the epidermis	Value range	Score
1. Epidermal parakeratosis	0-3	
2. Acanthosis	0-3	
3. Elongation of rete ridges	0-3	
4. Hypogranulosis	0-3	
5. Thinning of the suprapapillary plate	0-3	
6. Epidermal spongiosis	0-3	
Morphological Features of the dermis		
7. Dermal papillary oedema	0-3	
8. Tortuous papillary dermal blood vessels	0-3	
Cellular elements of inflammation in the epidermis		
9. Epidermal neutrophils	0-3	
10. Epidermal lymphocytes	0-3	
11. Collections of intraepidermal Mononuclear cells	0-3	
Cellular elements of inflammation in the dermis		
12. Dermal lymphocytes	0-3	
13. Dermal neutrophils	0-3	
Total histological score (THS)	0-39	
Total Morphological Score (TMS)	0-24	
Total Inflammatory Score (TIS)	0-15	
Additional Features		
1. Epidermal Thickness		mm
2. Presence of epidermal eosinophils	0-3	
3. Presence of dermal eosinophils	0-3	
4. Presence of Dermal mast cells	0-3	
5. PAS stain	-/+	
Comments		
Clinician's signature	Date:	

3. Trozak's histological grading system

Name of Study: _____

Slide Accession Number: _____

HISTOLOGIC GRADING SYSTEM FOR PSORIASIS

Microscopic Criteria		*Value/Criteria*	*Score*
1. Regular elongation of the rete ridge		1	
2. Club shaped rete ridges		2	
3. Elongation and edema of the dermal papillae		1	
4. Perivascular mononuclear infiltrate in the upper dermis of papillae		1	
5. Absent granular layer	a. focal	1	
	b. total	2	
6. Parakeratosis	a. focal	1	
	b. total	2	
7. Suprapapillary plate thinning		2	
8. Mitosis above basal cell layer		2	
9. Munro microabscesses		3	
10. Spongiform pustule		3	
	Score total:	19	
Epidermal Thickness			
Suprabasilar Mitosis Average per 8 HPF			

Comments: _____

_____

Investigators Signature_____ Date _____

Adapted from (Trozak, 1994) original paper.