Mucosal Associated Invariant T Cells and Insulin Resistance in Hidradenitis Suppurativa

A Thesis Presented to Trinity College, The University of Dublin for the Degree of Doctor of Medicine (M.D.)

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Declaration of Authorship

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Summary

Hidradenitis suppurativa (HS) is a chronic, inflammatory skin disease characterised by recurrent painful nodules, abscesses and draining sinus tracts. There is a paucity of effective treatments and it severely impairs patients’ quality of life\(^1\). The pathogenesis remains unclear, but emerging evidence suggests immune dysregulation and inflammation are key drivers of the disease\(^2\).

In addition, several studies have confirmed that patients with HS have a higher body mass index (BMI) compared to the general population\(^3\). It is known obesity is characterized by inflammation of the adipose tissue and this inflammation is a major driver of insulin resistance (IR)\(^4\) which, in turn, is a strong predictor of type 2 diabetes and the development of cardiovascular disease\(^5\). Interleukin (IL)-17 has been found to participate in this complex interplay between inflammation and metabolism\(^6\) and has also been implicated in HS pathogenesis, potentially explaining a link between the two.

Mucosal- associated invariant T (MAIT) cells are a novel subset of innate-like T cells. They are activated upon recognition of bacterial derivatives and can rapidly produce a milieu of cytokines including TNF\(\alpha\), IFN\(\gamma\) and IL-17\(^7\). The Retinoic acid receptor-related-\(\gamma\)t (ROR\(\gamma\)t ) is the key transcription factor in IL-17 producing T cells\(^8\) and is emerging as an important therapeutic target given its association with autoimmune diseases\(^9\). However, the implication of both MAIT cells and ROR\(\gamma\)t in HS remains unknown.
In light of this, my thesis reports two main studies which have HS as a central theme. I first sought to explore the relationship between IR and HS. Next, I investigated the role of IL 17 producing MAIT cells in the disease and examined the potential of targeting IL-17+ MAIT cells using a small molecule RORγt inhibitor.

To address my research questions, I established two well defined patient cohorts (94 patients and 15 patients). Firstly, I performed a prospective study to establish the prevalence of IR in our HS patients and to record the presence of its cutaneous manifestations. In the second cohort I prepared peripheral blood mononuclear cells (PBMC) and skin biopsies for flow cytometric analysis. MAIT cell cytokine production was determined by intracellular flow cytometry while MAIT cell RORγt expression in patients and controls was determined by transcription factor flow cytometry.

This thesis presents several important findings; there was a high frequency of IR in our HS cohort and these patients were more likely to be obese. It highlights how close observation of cutaneous signs may be an indicator of internal disease and reminds me why I pursued a career in Dermatology; we have the wonderful opportunity to diagnose with our eyes. Next, we made the novel observation that there is an accumulation of IL-17 producing MAIT cells in hidradenitis suppurativa lesions. Our results also provide supporting data for investigating RORγt small molecule inhibitors in the treatment of patients with H.S. Collectively, this work enhances awareness of HS and provides further insights into the understanding of its pathogenesis and novel treatments.
Preface

Under the guidance of my supervisors Professor Anne-Marie Tobin and Dr Andrew Hogan, I have been solely responsible for the research design, execution, analysis and presentation of data in this thesis.

Dr Andrew Hogan supervised and assisted my laboratory work in the Education and Research Centre and the "Obesity" laboratory in Maynooth University.

I obtained ethical approval, recruited patients, conducted all clinical assessments, drew blood and performed skin biopsies from patients where necessary.

Full and informed consent was obtained from all participants involved in this study.

Blood samples for insulin and glucose were analysed in Tallaght University Hospital laboratory.

I have performed all relevant statistical analysis on data collected.

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I am thankful to Maynooth University for the opportunity to engage in scientific research and I am tremendously grateful to everyone in the Obesity Laboratory for their patience, help and training.

I am grateful to my colleagues and the nurses in Tallaght University Hospital Dermatology Department for their support and assistance in carrying out this research. I am especially thankful to the patients for their participation and commitment to advancing Dermatology research.

Special thanks must go to my Aunt Colette and Uncle Patrick who I lived with while undertaking this MD.

Finally, I would like to express my deepest appreciation to my parents for everything they do.
For My Parents
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Glossary of Abbreviations

AGA Androgenetic Alopecia
AHRs Aryl Hydrocarbon Receptors
AN Acanthosis Nigricans
AS Ankylosing Spondylitis
AT Adipose Tissue
BAD British Association of Dermatology
BMI Body Mass Index
CVD: Cardiovascular Disease
DLQI Dermatology Life Quality Index
EHSF European Hidradenitis Suppurativa Foundation
FSC Forward Scatter
GLP-1 Glucagon-like peptide 1
HiSCR Hidradenitis Suppurativa Clinical Response
HOMA-IR Homeostasis Model of Insulin Resistance
HS Hidradenitis Suppurativa
IBD Inflammatory Bowel Disease
IGF Insulin Growth Factor receptors
IHS4 Initial HS Severity Score System
IL-1β Interleukin 1 beta
IL17 Interleukin -17
iNKT Invariant Natural Killer T
Interferon Gamma IFN-g
IR insulin Resistance
IR Insulin Resistance
MAIT Mucosal- associated invariant T cells
MHC Major Histocompatibility Complex
nAChRs Nicotinic Acetylcholine Receptors
OR odds ratio OR
PAPASH Pyogenic Arthritis Pyoderma Gangrenosum Acne
HS Hidradenitis Suppurativa
PASH Pyoderma Gangrenosum Acne HS
PBMC Peripheral Blood Mononuclear Cell
PCOS Polycystic Ovarian Syndrome
PGA Physician Global Assessment
RORγt Retinoic acid receptor-related-γt
RPMI Roswell Park Memorial Institute medium
SD Standard Deviation
SLE Systemic Lupus Erythematosus
SSC Side Scatter
T2DM Type 2 Diabetes Mellitus
TCR T cell receptor
TNF-alpha Tumour Necrosis Factor alpha
Treg regulatory T
WHO World Health Organization
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Chapter One: Introduction

1.1 Chapter Overview
This chapter provides an overview of HS and disease pathogenesis. Clinical aspects, treatment and co-morbid metabolic dysfunction are discussed. Finally, current knowledge on MAIT cells is reviewed focusing on their involvement in the pathogenesis of skin conditions and metabolic disease.

1.2 Hidradenitis Suppurativa

1.2.1 Definition
HS is defined as a ‘chronic, inflammatory, recurrent, debilitating, skin follicular disease characterized by painful nodules, abscesses, and sinuses in the apocrine Gland-bearing areas of the body, most commonly, the axillary, inguinal and anogenital regions10.

1.2.2 Epidemiology
Globally, estimates of HS prevalence vary widely depending on the method of data collection11 but it has been estimated at approximately 1–4% in the U.K. population12. Typical age of onset for the majority of patients appears to be their twenties13. HS is very rare before menarche, although paediatric cases have been reported14. There is a female predominance in western countries, whereas reports from Asia predominantly describe men15. A population-based study in the United States found
that the prevalence of HS in African American and mixed-race individuals was 3 times and 2 times higher than that of white individuals, respectively\textsuperscript{16}.

\textbf{1.2.3 Risk factors}

\textit{Genetics}

As with most chronic inflammatory diseases, a genetically based predisposition and environmental or lifestyle factors contribute to disease development\textsuperscript{15}. A positive family history is observed in \(~30\%\) of patients and the pattern of inheritance suggests an autosomal dominant trait. Heterozygous $\gamma$-secretase gene mutations have been found in patients with HS from China, Europe, and other locations\textsuperscript{17}.

\textit{Smoking}

Approximately 90\% of patients with HS are current or former smokers\textsuperscript{15}. However, the role of cigarette smoking in HS pathogenesis remains unclear and appears to be multifactorial. A recent review suggests tobacco smoke with many of its chemicals as well as nicotine promote the proinflammatory cytokines found in HS lesions, activate the nicotinic acetylcholine (nAChRs) and aryl hydrocarbon receptors (AHRs), and further suppress Notch signalling pathway contributing to the disease\textsuperscript{18}. All patients should be counselled on smoking cessation.
**Obesity**

In addition, 50% of patients with HS are obese, defined as a Body Mass Index > 30 kg/m^2^ which contributes to disease pathogenesis through subclinical inflammation, metabolic changes and increased friction in skin folds\(^1\).

**1.2.4 Clinical Features**

The typical clinical features include comedones (characteristically double), papules, pustules and inflammatory nodules in flexural areas. These progress to cysts, abscesses, sinus tracts and fistulae. Longstanding disease can result in fibrosis, dermal contractures, scarring and a consequent reduction in mobility. However, phenotypic variation is high, likely due to various underlying aetiologies that remain incompletely understood\(^2\). In addition to this “typical” presentation, Canoui-Poitrine and colleagues identified two atypical phenotypes: the “follicular” form characterized by follicular lesions, severe acne, and a predominance of men; and the “gluteal” form characterized by selective involvement of the gluteal area\(^3\). These phenotypes are usually more severe.

It has a seriously negative impact on patients' lives as symptoms include severe pain, as well as pruritus and is often accompanied by a chronic malodorous discharge (serous, purulent or blood-stained). Unsurprisingly, this leads to a marked degree of frustration, embarrassment, self-consciousness and depression\(^4\).
Other disease complications include fistula formation (affecting the genitourinary system and rectum), lymphoedema, anaemia and the development of squamous cell Carcinoma\textsuperscript{23}.

Furthermore, HS has also been reported in the literature to be a component of the autoinflammatory syndromes PASH (pyoderma gangrenosum, acne, suppurative hidradenitis) and its variant PAPASH, which have been reported to be of a more severe phenotype and accompanied by other systemic symptoms\textsuperscript{24}.

\subsection*{1.2.5 Diagnosis and Measurement of Disease Severity}

\textit{Diagnosis}

HS is a clinical diagnosis. It is based on consensus diagnostic criteria\textsuperscript{25} which require individuals to have:

| The presence of typical lesions; painful nodules, abscesses, sinus tracts, bridged scars or open comedones |
| Lesions occurring in at least one typical body location; axillae, groin, perineal region, perianal region, infra- and intermammary folds or buttocks |
| The disease must be chronic and recurrent |

\textbf{Table 1.1 British Association of Dermatology diagnostic criteria for HS}

As mentioned, HS has an extremely heterogenous clinical presentation and the diagnosis is frequently delayed. Kokolakis and colleagues found the average duration from manifestation of first symptoms until HS diagnosis was as long as 10.0 years\textsuperscript{26}.
Measurement of Disease Severity

Baseline disease severity in each skin region is often measured using the Hurley staging system. This staging system separates patients into three stages as follows:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Solitary or multiple, isolated abscess formation without scarring or sinus tracts</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Recurrent abscesses, single or multiple widely separated lesions, with sinus tract formation</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Diffuse or broad involvement, with multiple interconnected sinus tracts and abscesses.</td>
</tr>
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</table>

Table 1.2 Hurley Staging system

The Hurley system is static and was not designed as a dynamic score, and so other instruments are used to measure the efficacy of treatment. These include; the Sartorius Hidradenitis Suppurativa Score is made by counting involved regions, nodules and sinus tracts, the Physician Global Assessment (PGA) which is a six-point scale ranging from clear to very severe. More recently, the Hidradenitis Suppurativa Clinical Response (HiSCR) has been developed as an endpoint for clinical trials. It is defined as a ≥ 50% reduction in inflammatory lesion count (abscesses + inflammatory nodules), and no increase in abscesses or draining fistulas when compared with baseline. A Delphi voting procedure was recently conducted among the members of the European Hidradenitis Suppurativa Foundation (EHSF) which resulted in validating the novel Initial HS Severity Score.
System (IHS4) which dynamically assesses HS severity\textsuperscript{13}. The Dermatology Life Quality Index (DLQI) is also useful to assess the impact on daily life.

1.2.6 Treatment

The treatment of HS is notoriously challenging and not one treatment has proven beneficial to every patient. Moreover, clinical practice is variable and evidence-based treatment guidelines are lacking\textsuperscript{29}.

As previously mentioned, the literature supports an association with HS and both smoking and obesity. These risk factors are also associated with an increased risk of cardiovascular events and mortality. It is therefore pertinent to counsel patients on smoking cessation and weight loss. In addition, weight loss is associated with clinical improvement and we reported two cases of remission of HS following bariatric surgery\textsuperscript{30}.

For mild disease, topical clindamycin 1\% is a possible therapy, especially in the absence of abscesses. Intrallesional corticosteroid therapy can also be effective for isolated HS lesions\textsuperscript{31}. If there are several lesions and frequent exacerbations, systemic tetracyclines should be considered as per the BAD guidelines\textsuperscript{25}. Combined rifampicin and clindamycin for 10 weeks is considered as a second line systemic therapy for active HS\textsuperscript{32}.
Adalimumab is considered the first-line biologic therapy in HS due to the amount of high-level evidence available to support its use, followed by infliximab and anakinra as second- and third-line treatments, respectively\textsuperscript{29}. These agents have provided valuable options for those with severe refractory disease, however, for a considerable number of patients TNF-\(\alpha\) inhibitors are ineffective. Recent research has sought to identify additional treatment options that target cytokines central to the HS cascade\textsuperscript{33} and there are multiple studies reporting off-label novel biologics successfully managing even recalcitrant disease.

Two randomised, double-blinded, placebo-controlled phase 3 trials published on secukinumab have given hope to HS patients for additional medications to be approved for the disease, with 45\% of patients experiencing clinical improvement and a sustained response to treatment\textsuperscript{34}. Data from a phase 2, randomized trial evaluating bimekizumab, which inhibits both IL-17A and IL-17F, demonstrated clinically meaningful improvements across all outcome measures and a recent meta-analysis demonstrated its success in achieving significant improvement on HiSCR with an acceptable safety profile\textsuperscript{35,36}. Case series report success with Ixekizumab, another anti-IL-17 agent\textsuperscript{37} and an open-label cohort study of ten participants found significant improvements in pain, itch, quality of life, and depression with Brodalumab\textsuperscript{38}.

Case reports and meta-analysis have also shown efficacy for ustekinumab, an IL-12/23 inhibitor, in patients with moderate to severe, refractory HS \textsuperscript{39} and a phase II
trial has shown promise for the anti-IL23 inhibitor guselkumab\(^4\). A case series of five patients with moderate to-severe HS who were treated with tildrakizumab demonstrated an improvement in their HS abscess and nodule count at week 8 compared to their baseline\(^4\). In addition, there are case reports of severe Hurley III HS patients successfully with risankizumab, a humanised immunoglobulin G1 monoclonal antibody selective to the interleukin 23\(^2\), consolidating that IL-23 inhibitors may be a new promising treatment option for HS.

As mentioned, HS is associated with obesity and recent evidence supports the association between HS, metabolic syndrome and its components including insulin resistance. Metformin, an insulin-sensitizer, has proven beneficial in some cases, even though its mechanism of action in HS has yet to be fully elucidated, its efficacy appears to be due to lowered insulin resistance\(^4\). The BAD guidelines advise its use in HS patients with concomitant diabetes mellitus, and females with polycystic ovary syndrome or pregnancy\(^2\). Similarly, glucagon-like peptide 1 (GLP)-1 agonist therapy, used in the management of type 2 diabetes mellitus, dyslipidaemia and obesity, acts by potentiating glucose-dependent insulin secretion, reducing insulin resistance, inhibiting glucagon production and inducing satiety\(^4\). The successful use of GLP agonists has been described in psoriasis\(^4\), which has similar metabolic abnormalities to HS and Jennings et al report the use of liraglutide, in a case of recalcitrant HS leading to subsequent weight loss and rapid improvement in disease control\(^4\).
Spironolactone is an androgen antagonist that has been used in the treatment of acne, female pattern hair loss and hirsutism. Quinlan et al found it to be a safe and effective treatment option for female patients with HS\textsuperscript{47}.

Interestingly, the current BAD guidelines advise not to offer isotretinoin to treat people with HS unless they had concomitant moderate-to-severe acne, and we had previously reported a case series of eight patients who developed HS on commencement of this drug\textsuperscript{48}.

When considering surgery, again the evidence is lacking and based on case series and cohort studies with differing methodologies and outcome definitions\textsuperscript{29}. Generally, patients with severe disease may benefit most from surgery rather than medical interventions and radical wide excision of all involved skin and tissue is the only curative treatment\textsuperscript{49}.

With increasing research into the disease, there is also more work into the development of new treatments for this debilitating disease and clinical trials are currently underway to hopefully increase the variety of treatment options for our patients.

\textbf{1.2.7 HS and Metabolic Dysfunction}

There is an emerging consensus that inflammation links obesity, metabolic dysfunction and inflammatory disorders\textsuperscript{50,51}. 
A recent systematic review and adjusted meta-analysis found that there was a significant and independent association between HS and metabolic syndrome\(^5\). A primary characteristic of metabolic syndrome includes obesity and the relationship between HS and obesity is well established. Revuz et al found an increased risk of HS by 1.12 for each unit increase in BMI\(^3\) and Kromann and colleagues found the point prevalence of obesity in a group of patients undergoing weight reduction surgery was almost 20\(^%\)\(^5\).

However, it remains unclear whether metabolic syndrome is triggered by the chronic inflammatory nature of HS or whether patients with metabolic syndrome are predisposed to HS\(^5\). For example, the obese have a larger surface area of skin resulting in more friction from skin folds and the humid microclimate in skin folds favours bacterial growth, influencing HS pathogenesis\(^5\). In addition, obesity is characterized by a chronic low-grade inflammation of the visceral adipose tissue and this inflammation is a major driver of insulin resistance (IR)\(^4\). In a cross-sectional, case–control study Vilanova et al found a significantly higher prevalence of IR in their HS patients compared to controls\(^5\). These authors note that even after adjustment for BMI, age and sex, the HOMA-IR value remained significantly higher in HS patients suggesting that HS itself may be an independent risk factor for the development of IR\(^5\).

Additionally, there is increasing evidence in the literature of improvements in HS occurring following weight-reduction in patients with obesity further supporting this
pathogenic link. I reported two cases of remission of HS following bariatric surgery\(^\text{30}\). Interestingly, our patients also had a marked improvement in insulin resistance, and it is possible that some of the clinical improvement observed may be associated with reduced levels of insulin.

1.2.8 HS Pathogenesis and Immune Dysfunction

HS is a complex disorder and its pathogenesis remains unclear\(^\text{56}\). Emerging evidence has highlighted significant immune dysregulation and inflammation as key drivers of the disease\(^\text{2}\).

It appears to start around the hair follicle; the primary event is follicular occlusion due to infundibular hyperkeratosis of the terminal follicles and hyperplasia of the follicular epithelium\(^\text{57}\). This results in collections of cellular debris which presents as painful, inflamed nodules and cysts. There is subsequent rupture of the follicle, sinus tract formation and eventually, scarring. In addition, this disruption of the hair follicle produces a significant inflammatory response and influx of inflammatory cytokines\(^\text{58}\). Several studies have identified these pro-inflammatory cytokines as possible contributors to HS, notably TNF-alpha, IL-1beta and IL-17\(^\text{59-61}\), which is further supported by the clinical responsiveness of HS patients to TNF-alpha inhibitors, corticosteroids and immunosuppressive agents\(^\text{62-65}\).

Immune dysregulation is definitely implicated in the disease pathogenesis, but one population that has yet to be studied are Mucosal-associated invariant T (MAIT) cells.
1.3 Mucosal associated invariant T (MAIT) cells

1.3.1 What are MAIT cells and why are they relevant?

The immune system is traditionally made up of two parts: the innate (general) immune system and the adaptive (specialised) immune system. Conventional T and B cells of the adaptive immune system induce specific responses and provide long-term memory against microorganisms, whereas the innate immune system allows immediate recognition of pathogens and moulds adaptive immune responses; mucosal-associated invariant T (MAIT) cells are unique innate-like T cells that serve as a bridge between the two.

They were first described in 1993 by Porcelli et al., and later, in 1999, their findings were further confirmed by another influential study by Tilloy and colleagues who showed that this new type of T cell subset was present in humans, mice, and cattle, indicating conservation between mammalian species highlighting their importance.

However, identifying the critical role played by these cells has not proved straightforward, perhaps because these cells perform several distinct functions.

These cells are characterised by their expression of the invariant T cell receptor (TCR)α chain, Vα7.2 in humans, or Vα19 in mice. In addition, they express high levels of the C-type lectin CD161, as well as CD3 and CD8. The CD4⁺/CD8⁻ MAIT cell population has recently been described as a functionally distinct MAIT cell subset, producing higher levels of IL-17 and lower levels of IFN-γ.
MAIT cells are remarkably abundant in human tissues; they constitute 2-10% of peripheral T cells and are found in the gut\textsuperscript{72,73}, lungs\textsuperscript{74}, adipose tissue\textsuperscript{75} and most markedly in the liver, where they account for up to 50% of all T cells\textsuperscript{76}.

A defining feature of MAIT cells are they are activated when their invariant TCR recognize microbially-derived vitamin metabolites presented on the major histocompatibility complex (MHC) like molecule MR1\textsuperscript{77}. MAIT cells can also be activated in a T cell receptor independent manner, via cytokine stimulation\textsuperscript{78}. This means that MAIT cell activation is not solely reliant on TCR ligation alone and the cells can respond to a wider set of stimuli\textsuperscript{70}. (Figure 1.1).
MAIT cells can be activated in a MR1 dependent manner (1) where bacterial ligands are presented by antigen presenting cells (APC) on MR1 and recognised by the invariant Vα7.2 TCR resulting in the production of cytokine and lytic molecules. MAIT cells can also be activated in a MR1 independent manner (2) by inflammatory cytokine such as IL-12 and IL-18 which are produced by innate cells in the presence of viral or bacterial infection, again resulting in the production of effector molecules.

MAIT cells are early responding T cells that are capable of rapidly producing multiple cytokines upon activation such as IFN-gamma, TNF-alpha and IL-17. This feature makes MAIT cells potent anti-bacterial and viral effectors but has also implicated them in the pathogenesis of many several diseases including rheumatoid arthritis, obesity and psoriasis. Their role in H.S is currently unknown.
1.3.2 Pathogenic role of IL-17-producing MAIT cells

Human and murine MAIT cells can express retinoic acid receptor-related-γt (RORγt), the key transcription factor required for Th17 cell differentiation and for the production of the IL-17 cytokines by innate and adaptive immune cells.83

Th17 cells have been implicated in a number of inflammatory diseases84 and recently Moran and colleagues revealed that the frequencies of the Th17 cells and regulatory T (Treg) cells are elevated in HS lesions85.

Furthermore, Kelly et al demonstrated that CD4⁺ T cells produce IL-17 in HS and suggested that the IL-17 pathway may be important in HS pathogenesis59. In concordance with this, Lima et al reported IL-17-producing cells are present in lesional and perilesional HS skin and may contribute to the initiation of the inflammatory processes86. In addition, another group reported significantly elevated IL-17 serum levels in patients with HS compared to controls60.

Given this association with autoimmune diseases and its critical role in the generation of IL-17-producing T cells, RORγt inhibition is emerging as an important therapeutic target9.

Small molecular inhibitors of RORγt were only first described in 2011 by Huh and colleagues who demonstrated efficacy in murine models of autoimmune inflammation8. Since then, several studies have investigated the targeted inhibition of RORγt as an alternative to anti-IL-17 biological therapy9. In spondylarthritits patients RORγt inhibition
selectively targeted IL-17 producing iNKT and γδ-T cells. Similarly, Guendisch and colleagues demonstrated positive effects of RORγt inhibition in murine models of arthritis. To date the implication of RORγt in HS remains unknown.

### 1.3.3 MAIT cells in Chronic Disease

The role of MAIT cells in chronic disease is emerging with dysregulated MAIT cell cytokine profiles being reported in several chronic inflammatory diseases.

MAIT cells are noted to accumulate in the tissue of several other inflammatory diseases. Carolan et al investigated MAIT cells’ role in obesity and found they are enriched in human adipose tissue. In addition, MAIT cells have been shown to migrate into the synovial fluid in Rheumatoid Arthritis and ankylosing spondylitis. In Sjögren syndrome MAIT cells were found to infiltrate the salivary glands and are reported to be increased in the inflamed tissue in Inflammatory bowel disease (IBD).

Several studies have recently detailed the presence of MAIT cells in human skin. Teunissen et al found IL-17A-producing CD8 MAIT cells were predominantly in psoriatic plaques and were almost absent in healthy skin. Li et al found the proportion of MAIT cells infiltrating the skin to be selectively increased in dermatitis herpetiformis, suggesting a role for MAIT cells in the pathogenesis of this disease. In 2019, Constantinidies also reported the presence of MAIT cells in both murine and human skin, describing a wound healing function.
Several studies have described altered MAIT cells in children and adults with obesity. Carolan et al found that in adults with obesity, there was an increase in the frequency of IL-17–producing MAIT cells and the change in MAIT cell frequency was greater in individuals with uncontrolled HbA1c suggesting a possible role for them in the pathogenicity of obesity. In 2020, Toubal and colleagues provided evidence which directly implicated MAIT cells in the development of metabolic dysregulation using MAIT cell deficient murine models of obesity. Most recently, Bergin and colleagues provided more evidence that MAIT cells, and in particular the IL-17 producing subset can directly disrupt insulin signalling and drive insulin resistance.

1.4 Conclusion
In summary, HS is a chronic, inflammatory skin disease of the hair follicles, resulting in painful lesions of apocrine-bearing skin. The underlying pathogenic mechanisms are complex and not fully understood. The cutaneous changes start around the hair follicles and involve activation of cells of the innate and adaptive immune systems, with pivotal roles for pro-inflammatory cytokines such as IL-17. Furthermore, MAIT cells are emerging as significant players in the human immune system. They possess attributes of both innate and adaptive immunity and are capable of rapid cytokine secretion including IL-17. However, their role in HS has not been investigated. In addition, there is a paucity of effective treatments for HS and RORγt inhibition represents a potential novel therapeutic strategy. Interestingly, obesity and insulin resistance are strongly associated with HS and have also been shown to impact MAIT cell frequency and function.
1.5 Aims of this thesis

The main aims of this thesis were therefore to examine MAIT cells in HS as well establish the frequency of IR in our HS cohort.

Specific aims included the following:

- Establish the prevalence of IR in our HS patients
- Assessing frequencies of MAIT cells in HS & control blood
- Assessing frequencies of MAIT cells in lesions & adjacent skin
- Cytokine profiling MAIT cells in blood & skin of HS
- Targeting RORγT + MAIT cells in HS
Chapter Two: Materials and Methods

2.1 Ethics

All studies were conducted in accordance with the Helsinki Declarations and ethical approval was granted by the Joint Research Ethics Committee of Tallaght University Hospital and St James’s Hospital, for all parts of this study.

2.2 Study design

All studies were prospective cohort studies, performed over a 24-month period from March 2017-March 2019.

2.3 Study cohorts

Two cohorts were recruited for studies included in this thesis

a) A cohort of HS patients for the study of insulin resistance and its cutaneous findings

b) A cohort of patients with HS for the investigation of MAIT cells

I recruited and assessed all subjects, ensuring consistency in evaluation.
2.3.1 Cohort of HS patients for study of insulin resistance in HS

This study involved recruiting sequential patients attending the Dermatology Outpatient Department in Tallaght University Hospital, Dublin 24, with a diagnosis of Hidradenitis Suppurativa (HS) and aged over 18 years of age.

All subjects met the British Association of Dermatologists (BAD) diagnostic criteria\textsuperscript{25} for the diagnosis of HS. The diagnosis was performed by a Dermatologist. The criteria are as follows:

| The presence of typical lesions; painful nodules, abscesses, sinus tracts, bridged scars or open comedones |
| Lesions occurring in at least one typical body location; axillae, groin, perineal region, perianal region, infra- and intermammary folds or buttocks |
| The disease must be chronic and recurrent |

Table 2.1 British Association of Dermatologists (BAD) diagnostic criteria\textsuperscript{25} for the diagnosis of HS

Exclusion criteria from the study included; Age <18 years, co-morbid inflammatory conditions outside of HS, patients who lack capacity to give consent.

All participants were provided with an information leaflet about the study and given at least 24 hours to read the information, ask questions and to consider voluntary participation in the study. Once agreeable to partake, informed written consent was obtained from all participants.
Upon recruitment, each patient was assigned a unique numerical and alphabetical identifier code (e.g. HS1) to ensure all clinical and research data was anonymised. All participant data was stored in both a locked office and on a password protected database on a personal computer in the Trinity Education Centre in Tallaght University Hospital.

Each participant had a comprehensive clinical assessment focusing on evidence of the cutaneous manifestations of insulin resistance. Following this metabolic was profiling conducted. Fasting venous blood samples for glucose and insulin were collected in lithium heparin tubes and sent to the hospital laboratory. (Figure 2.1)

2.3.2 Cohort of HS patients for study of MAIT cells in HS

This study involved recruiting sequential patients attending the Dermatology Outpatient Department in Tallaght University Hospital, Dublin 24, with a diagnosis of Hidradenitis Suppurativa (HS) and aged over 18 years of age.

All subjects met the British Association of Dermatologists (BAD)diagnostic criteria\(^{25}\) for the diagnosis of HS as previously mentioned. Again, the diagnosis was performed by a Dermatologist.

Exclusion criteria included; Age <18 years, active or recent infections in last 4 weeks, co-morbid inflammatory conditions outside of HS, patients on anti-inflammatory
medications, immunosuppression usage in last 6 months (as this may affect the population of innate cells in the skin) and patients who lack capacity to give consent.

Following informed consent, and clinical assessment venous blood samples were collected in two lithium heparin lined tubes for peripheral blood mononuclear cell (PBMC) isolation.

Two 6mm punch biopsies were obtained; one from the leading edge of an active nodule and a second from uninvolved skin 10cm from the lesion. The area to be sampled was injected with local anaesthetic (2% xylocaine and adrenaline) and once numb the biopsy was taken. The site was sutured with two dissolvable sutures. The biopsies were placed in Roswell Park Memorial Institute (RPMI) medium and was taken to the Human Health Institute, Department of Biology, Maynooth University, Maynooth.

A cohort of healthy controls were also recruited, and consisted of healthy hospital based volunteers with no known skin or inflammatory disorders. Other exclusion criteria were identical to those outlined above.

Similar to the HS patients, venous blood samples were collected in two lithium heparin lined tubes for peripheral blood mononuclear cell (PBMC) isolation. No skin was obtained from our healthy controls. (Figure 2.2)
A Study to Investigate Insulin Resistance in Hidradenitis Suppurativa

Sequential patients attending the Dermatology Department

<table>
<thead>
<tr>
<th>INCLUSION CRITERIA</th>
<th>EXCLUSION CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 years or older</td>
<td>Age &lt;18 years</td>
</tr>
<tr>
<td>Diagnosis of HS</td>
<td>Patients who lack capacity to give consent</td>
</tr>
</tbody>
</table>

CLINICAL EXAMINATION
Basic Demographics
Anthropometric measurements
Cutaneous signs of Insulin Resistance

BLOOD SAMPLE

Homeostasis model assessment for insulin resistance (HOMA-IR)

\[
\text{fasting plasma insulin} \times \text{fasting glucose} = 22.5
\]

A score > 2.5 was defined as insulin resistant

Figure 2.1 Schematic of recruitment and assessment of cohort of HS patients with insulin resistance and its cutaneous findings
A Study to Investigate MAIT cells in HS

Sequential patients attending the Dermatology Department

<table>
<thead>
<tr>
<th>INCLUSION CRITERIA</th>
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</tr>
</thead>
<tbody>
<tr>
<td>18 years or older</td>
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<tr>
<td>Diagnosis of HS</td>
<td>Patients who lack capacity to give consent</td>
</tr>
<tr>
<td></td>
<td>Active or recent infections in last 4 weeks</td>
</tr>
<tr>
<td></td>
<td>Co-morbid inflammatory</td>
</tr>
<tr>
<td></td>
<td>Patients on anti-inflammatory medications</td>
</tr>
<tr>
<td></td>
<td>Immunosuppression usage in last 6 months</td>
</tr>
</tbody>
</table>

CLINICAL EXAMINATION

BLOOD AND SKIN SAMPLES

Sample collection

Multi-colour flow cytometry

Ex-vivo treatment

Figure 2.2 Schematic of recruitment and assessment of cohort of HS patients and healthy controls to investigate MAIT cell frequency and function
2.4 Clinical Assessment of subjects with HS

Each participant had a comprehensive clinical assessment. A succinct medical history was conducted for each participant to ensure suitability for the study. A proforma was devised to record demographic and other relevant data of patients included (Appendix).

Patients’ basic demographic data including age and sex were recorded, as well as duration of disease, smoking history and current treatment.

Anthropometric measurements were taken to calculate body mass index (BMI). BMI was calculated for all patients recruited using the formula:

\[
BMI = \frac{\text{Weight (kg)}}{\text{Height (m)}^2}
\]

Body mass indices were classified using the World Health Organization (WHO) definition as below:

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>≤ 18.49</td>
</tr>
<tr>
<td>Healthy</td>
<td>18.5-24.9</td>
</tr>
<tr>
<td>Overweight</td>
<td>25-29.9</td>
</tr>
<tr>
<td>Obese</td>
<td>≥ 30</td>
</tr>
<tr>
<td>Morbid obesity</td>
<td>≥ 40</td>
</tr>
</tbody>
</table>

Table 2.2 BMI Classification
All patient’s physical examination focused on Hurley stage and the presence of the cutaneous manifestations of insulin resistance which includes acanthosis nigricans (Figure 2.3), acrochorda (Figure 2.3), acne, hirsutism, androgenic alopecia and tinea incognito.

Figure 2.3 Acrochorda, Acanthosis Nigricans and Hurley Stage 2 HS
Hurley stage II HS patient with confirmed insulin resistance on blood sampling with evidence of hyperpigmented velvety changes in the axilla consistent with acanthosis nigricans and also acrochorda

The clinical severity of HS was assessed by the Hurley Score. This staging system separates patients into three stages as follows:

| Stage 1     | Solitary or multiple, isolated abscess formation without scarring or sinus tracts |
Stage 2  Recurrent abscesses, single or multiple widely separated lesions, with sinus tract formation
Stage 3  Diffuse or broad involvement, with multiple interconnected sinus tracts and abscesses.

Table 2.3 Hurley Staging System

2.5 Insulin Resistance

Fasting blood samples were taken for the measurement of insulin and glucose. Serum insulin concentrations were determined in the hospital laboratory by automated chemiluminescence immunoassay on a Tosoh platform (Tosoh, Tokyo, Japan). Plasma glucose concentrations were quantified with a hexokinase method (Roche Diagnostics). See Table 2.4 for reference ranges.

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Measured using the enzymatic reference method with hexokinase</td>
<td>2.8-6.0 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>Measured using the Elecsys insulin assay</td>
<td>2-25 mU/L</td>
</tr>
</tbody>
</table>

Table 2.4 Insulin and Glucose Hospital Reference Ranges
Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the formula fasting plasma insulin \( \times \) fasting glucose /22.5. A score > 2.5 was defined as insulin resistant.

2.6 Lab based experiments

2.6.1 Peripheral Blood Mononuclear Cell Isolation

Peripheral Blood Mononuclear Cell (PBMC) were isolated by density centrifugation from fresh venous blood samples. The 20ml of venous blood collected in lithium heparinised vacutainers was processed within 4 hours to isolate Peripheral Blood Mononuclear Cell Isolation (PBMCs). Standard density gradient centrifugation over Lymphoprep technique was used to obtain PBMCs. 20ml of venous blood was diluted in 1:2 ratio with phosphate buffered solution (PBS) and then slowly layered into 15ml of Lymphoprep. Samples were then centrifuged at 1600 RPM (450 RCF) for 20 minutes with fast acceleration and slow deceleration. The PBMC layer was then extracted by pipette (Figure 2.4) and washed in 50ml of PBS and centrifuged at 800G for 5 minutes. A final wash was performed, and the solution was centrifuged again at 500G for 10 minutes. The supernatant was removed, and remaining cell pellet was resuspended in 10ml R10 (RPMI + 10% FBS + supplements).
2.6.2 Infiltrating Lymphocytes

CD45+ skin resident cells were isolated from 6mm punch biopsies. Skin samples of both lesional and non-involved skin were incubated separately with 5mg/ml of type IV collagenase for 3 hours at 37 degrees. Following physical dissociation, the individual skin biopsies passed through an ultra-filter. The samples were then washed with 10ml of R10 and passed through the filter again. The resultant sample was centrifuged at 500G for 10 minutes. The cell pellet was resuspended in 1ml of R10 ready for cell count.

2.6.3 Cell count

PBMCs were diluted 1:20 with trypan blue exclusion which stains dead cells blue. The solution was loaded on a haemocytometer and examined immediately under a light microscope. The following formula was used to calculate the number of viable cells per mL of culture:

\[
\text{Number of cells counted per square millimetre \times dilution factor \times 10^4} = \text{cells/mL culture}
\]
The PBMCs were then re-suspended in R10 media to yield a final concentration of 1x10^6 cells/ml for further analysis. The same was performed for infiltrating lymphocytes for involved and non-involved skin.

### 2.6.4 MAIT Cell Cytokine Stimulation

Single cell suspensions of PBMCs and infiltrating lymphocytes from involved and un-involved skin were plated in at 1x10^5 cells/ml in 96-well tissue culture plate (Figure 2.5)

The conditions used were:

(A) Basal sample, unstimulated cells in RPMI alone

(B) Combination of TCR microbeads and IL-12/IL-18 which stimulates T cells

(C) PMA cocktail which opens calcium channels creating non-specific cell stimulation
Figure 2.5 Tissue Plate
MAIT cells were cultured in 96-well round-bottom plates

A protein transport inhibitor (Monensin/Brefeldin A), was added and the cells were placed in an incubator at 37°C and were stimulated for 18 hours. After which cells were investigated for intracellular cytokine production by flow cytometry.

Figure 2.6 MAIT cell cytokine stimulation

2.6.5 Extracellular staining

Following the 18-hour stimulation, MAIT cell staining (1 x 10⁶ PBMC) was performed using specific surface monoclonal antibodies (All Miltenyi Biotec) namely; CD3, CD161, CD8 and TCRα7.2 (Figure 2.6).
Figure 2.7 Extracellular staining
The individual cell suspensions from blood and skin experiments were labelled with fluorescent monoclonal antibody cell surface antigens CD3, CD8, CD161 & TCR Vα7.2

2.6.6 Intracellular staining
In order to identify the cytokine profile of MAIT cells we carried out intracellular cytokine staining for IL-17 and IFN-γ. PBMCs were fixed with 1% paraformaldehyde and then permeabilised using Saponin. They were then labelled with fluorescent monoclonal antibodies to IL-17 and IFNγ.

2.6.7 Flow cytometry
Cell populations were acquired using a using 14 colour Attune NXT flow cytometer and analysed using FlowJo software (Treestar). Results are expressed as a percentage of the parent population as indicated and determined using flow minus-1 (FMO) and unstained controls.
2.6.8 MAIT cell RORγt analysis

MAIT cell RORγt expression in patients and controls was determined by transcription factor flow cytometry. PBMC, CD45+ skin cells or expanded MAIT cells were activated as previously described for 18hrs before determining RORγt expression using a specific RORγt mAb (BD biosciences) and transcription factor staining kit (Biolegend). To assess the effect of RORγt inhibition on IL-17 production the specific inhibitor SR1001 (Tocris) was added at a final concentration of 5μM before stimulation and intracellular cytokine staining. FMO and unstimulated PBMC cells were used as negative controls.
2.8 Statistical analysis

Statistical analysis was completed using Graph Pad Prism 6 Software (USA). Data is expressed as standard error of the mean (SEM). Groups were compared using unpaired student t-test when comparing a single variable between two different groups with normal distribution. Mann Whitney U test was used when comparing a single variable in two samples that were not normally distributed. Analysis across 3 or more groups was performed using ANOVA. P values were expressed with significance set at <0.05.
Chapter Three: Insulin Resistance in Hidradenitis Suppurativa

3.1 Introduction

Several studies have confirmed that patients with HS have a higher Body Mass Index (BMI) compared to the general population. Revuz et al found an increased risk of HS by 1.12 for each unit increase in BMI\(^3\). In turn, obesity is characterized by a chronic low-grade inflammation of the visceral adipose tissue and this inflammation is a major driver of insulin resistance (IR)\(^4\). IR is a strong predictor of type 2 DM and the metabolic changes induced by IR may contribute to the development of accelerated atherosclerosis and CV disease\(^9\). One study observed that IR prevention may reduce myocardial infarction risk by as much as 42% in young adults\(^99\).

Insulin also has an important role in the homeostasis and physiology of the skin; the two main target cell types in skin diseases, keratinocytes and fibroblasts, both have insulin receptors and insulin growth factor (IGF) receptors\(^100\). Insulin is therefore thought to induce skin changes through activating these receptors in these cells, stimulating their proliferation. In addition, hyper-insulinaemia can also influence sex steroid production and increase free testosterone\(^101,102\). It is therefore not surprising that IR is associated with a wide variety of cutaneous manifestations including acanthosis nigricans (Figure 2.3), acrochorda (Figure 2.3) and tinea incognito\(^102\).
Polycystic Ovarian Syndrome (PCOS) is another manifestation of IR and notably is more common in patients with HS\textsuperscript{52}. Cutaneous signs of PCOS include acne, hirsutism and androgenic alopecia.

In light of this, the aim of this prospective cohort study was to establish the prevalence of IR in our HS patients and, for the first time to my knowledge, to assess whether its cutaneous manifestations were present on examination.

### 3.2 Results

#### 3.2.1 Study subjects

In all, 94 patients with HS were recruited. The majority were young (Mean age 35 ± 11 years), obese females (79%, n=74). Baseline characteristics of the study population are presented in Table 3.1.

#### 3.2.2 Prevalence of Insulin Resistance

Seventy-one percent (n=67) were insulin resistant; HOMA-IR 5.5±3.8. Those who were insulin resistant had a significantly higher BMI (Table 3.1). Notably, there was a significantly higher proportion of males in non-insulin resistant group. Both groups were similar in age.
<table>
<thead>
<tr>
<th>Participants</th>
<th>Insulin Resistant HS n=67 (Mean ± Sd)</th>
<th>Non-Insulin Resistant HS n=27 (Mean ± Sd)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35±9.7</td>
<td>34±13.3</td>
<td>0.298</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>9</td>
<td>0.075</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>37±7.5</td>
<td>28±6.4</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Overweight</td>
<td>8 (28.5±1.18)</td>
<td>10 (22.8±1.47)</td>
<td>0.018</td>
</tr>
<tr>
<td>Obese</td>
<td>40 (34.2±3.3)</td>
<td>7 (33.2±3.5)</td>
<td>0.018</td>
</tr>
<tr>
<td>Morbid obese</td>
<td>18 (46.97±5.5)</td>
<td>2 (43.2 ± 2.97)</td>
<td>0.4127</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.5±3.8</td>
<td>1.4±0.74</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

**Table 3.1 Baseline characteristics of the study population**

Values are expressed as mean SD or median (interquartile range) as appropriate. BMI, body mass index; HOMA-IR, Homeostatic model assessment for insulin resistance. The odds ratio (OR), its standard error and 95% confidence interval are calculated according to Altman, 1991.
3.2.3 Cutaneous signs of Insulin Resistance

All insulin-resistant patients had cutaneous signs of IR on examination. Thirty-nine percent had acrochorda and 25% had acanthosis nigricans. Twenty-eight percent of the group had known PCOS and the majority had cutaneous signs reflecting this; 57% had acne, 27% hirsutism and 21% androgenic alopecia. The presence of acne or hirsutism was not statistically significant. Of interest, none had tinea incognito.

<table>
<thead>
<tr>
<th>Cutaneous Signs of Insulin Resistance</th>
<th>Insulin Resistant HS n (%)</th>
<th>Non-Insulin Resistant HS n (%)</th>
<th>P Value</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrochorda</td>
<td>26 (39)</td>
<td>2 (7)</td>
<td>0.0077</td>
<td>7.9268</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(95% CI 1.7306 to 36.3090)</td>
</tr>
<tr>
<td>Acanthosis Nigricans</td>
<td>17 (25)</td>
<td>0</td>
<td>0.0426</td>
<td>19.0594</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(95% CI 1.1033 to 329.2629)</td>
</tr>
<tr>
<td>Tinea Incognito</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acne</td>
<td>38 (57)</td>
<td>11 (40)</td>
<td>0.3147</td>
<td>1.9060</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(95% CI 0.7694 to 4.7215)</td>
</tr>
</tbody>
</table>
Table 3.2 Comparison of Cutaneous signs of insulin resistance noted on examination in patients with Insulin Resistant HS and non-Insulin Resistant HS. Values are expressed as mean SD or median (interquartile range) as appropriate. BMI, body mass index; HOMA-IR, Homeostatic model assessment for insulin resistance. The odds ratio (OR), its standard error and 95% confidence interval are calculated according to Altman, 1991.

<table>
<thead>
<tr>
<th>Androgenic Alopecia</th>
<th>14 (21)</th>
<th>1 (4)</th>
<th>0.0697</th>
<th>6.8679</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8560</td>
<td>55.102</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3 Discussion

This study demonstrates a high frequency of IR in our HS cohort, which is in accordance with other reports. Özkur et al demonstrated that patients with HS have a significantly higher incidence of IR compared with an age, sex and BMI-matched control group\textsuperscript{103}. In a cross-sectional, case–control study Vilanova et al found a significantly higher prevalence of IR in their HS patients compared to controls. These authors note that even after adjustment for BMI, age and sex, the HOMA-IR value remained significantly higher in HS patients suggesting that HS itself may be an independent risk factor for the development of IR\textsuperscript{55}.

In this study the patients that were insulin resistant had a significantly higher BMI than those with a normal HOMA-IR. This is not surprising as it has long been established that obesity is closely associated with an increased risk of metabolic diseases.
including IR. In addition, metabolic disorders including obesity and metabolic syndrome are the most commonly associated conditions observed in patients with hidradenitis suppurativa\textsuperscript{19}. There is also emerging consensus that inflammation links obesity, metabolic dysfunction and inflammatory disorders \textsuperscript{50,51}.

Indeed, the question arises, is IR the driving factor of HS rather than being a secondary event as a result of chronic inflammation and obesity? High circulating levels of insulin are associated with excess sweating, particularly post-prandially, which may promote occlusion and maceration of axillae and inguinal creases. Obesity results in increased friction in skin folds, both contributing to HS pathogenesis suggesting IR as a driver of disease. Metformin, an insulin-sensitizer, has proven beneficial in some patients, even though its mechanism of action in HS has yet to be fully elucidated, its efficacy appears to be due to lowered insulin resistance\textsuperscript{44} again implicating IR in disease pathogenesis.

There was a significantly higher proportion of males in the non-insulin resistant group. It is known the majority of patients reported to have HS in western countries are women\textsuperscript{15}, but that does not explain why more women are insulin resistant in our cohort. Karagiannidis et al comment that the role of androgens and sexual hormones in the pathogenesis of the disease remains obscure\textsuperscript{104} but, the common comorbidity with metabolic syndrome points to possible interactions between endocrinological and metabolic alterations in the development of HS.

Notably, all patients who were insulin resistant in this study had cutaneous stigmata on examination; namely acanthosis nigricans (AN), acrochorda and androgenic alopecia. This is consistent with what is found in the most recent literature\textsuperscript{105}.,
however, studies investigating the relationship between metabolic alterations such as insulin resistance (IR) and dermatologic conditions are limited.

In this study, patients with AN were 19 times more likely to have insulin resistance. This is supported by Hud et al who reported acanthosis nigricans is a reliable cutaneous marker of hyperinsulinemia in obese individuals\textsuperscript{106}. Similarly, those with acrochorda on examination were almost 8 times more likely to have IR. In concordance with this finding, Tamega et al. report the presence of multiple skin tags was strongly associated with insulin resistance irrespective of other risk factors\textsuperscript{107}.

There are opposing reports regarding the relationship between androgenetic alopecia and insulin resistance, although insulin is suggested to play a role in the regulation of cutaneous androgen metabolism and hair-growth cycle\textsuperscript{108}. Nabaie et al. did not find an association between IR and AGA\textsuperscript{109} while Matilainen et al. reported a strikingly increased risk of hyperinsulinemia and IR-associated disorders in men with early onset of androgenetic alopecia (<35), compared with age-matched controls, supporting the hypothesis that early alopecia could be a clinical marker of IR\textsuperscript{110}.

Nonetheless, these skin manifestations of IR offer a reliable, straightforward, and real-time way to detect insulin resistance, to ultimately trigger an adequate metabolic evaluation and timely treatment to decrease diabetes and its associated disease burden\textsuperscript{105}.

The strength of the present study is that it was the first to evaluate cutaneous signs of IR in HS patients. However, there are some inherent limitations. Obesity is a major
confounder for IR, and we did not control for age, sex or BMI. It would also be of interest to assess co-morbid conditions associated with the metabolic syndrome. A record of their current treatment would be of relevance as some treatments may augment insulin resistance\textsuperscript{55}. However, despite these limitations, this study provides important insights into the association of HS and IR that may have clinical implications for the overall management of these patients.

3.4 Conclusion

In conclusion, this study found a high prevalence of IR and obesity in our HS patients. It highlights how close observation of cutaneous signs may be an indicator of internal disease and awareness of the cutaneous stigmata of IR should prompt clinicians to investigate their HS patients for metabolic abnormalities.

3.5 Key findings

- There is a high frequency of IR in our HS cohort
- Those who were insulin resistant were more likely to be obese
- Cutaneous manifestations of IR include acrochorda, acanthosis nigricans and androgenic alopecia
- Evidence of the cutaneous stigmata of IR in obese patients should prompt clinicians to investigate their HS patients for metabolic abnormalities and treat accordingly
- Close observation of cutaneous signs may be an indicator of internal disease
Chapter Four: Investigation of MAIT cells in Hidradenitis Suppurativa

4.1 Introduction

Hidradenitis suppurativa (HS) is a chronic, inflammatory skin disease characterized by recurrent painful nodules, abscesses and draining sinus tracts. There is a paucity of effective treatments and it severely impairs patients’ quality of life\textsuperscript{1}. The pathogenesis remains unclear, but emerging evidence has highlighted significant immune dysregulation and inflammation as key drivers of the disease\textsuperscript{2}.

Mucosal- associated invariant T (MAIT) cells are a novel subset of innate-like T cells. They are activated upon recognition of bacterial derivatives and rapidly produce a milieu of cytokines such as IL-17\textsuperscript{7}. Their implication in HS remains unknown.

The Retinoic acid receptor-related-\gamma (ROR\gamma ) is the key transcription factor in IL-17 producing T cells\textsuperscript{8}. It is emerging as an important therapeutic target given its association with autoimmune diseases\textsuperscript{9}.

As neither MAIT cells or ROR\gamma have not been studied in the context of H.S the focus of this study was to investigate their role in the disease and examine the potential of targeting IL-17+ MAIT cells using a small molecule ROR\gamma inhibitor.
4.2 Aims

- Assessing frequencies of MAIT cells in HS & control blood
- Assessing frequencies of MAIT cells in lesions & adjacent skin
- Cytokine profiling MAIT cells in blood & skin of HS
- Targeting ROR\(_{gamma}\) + MAIT cells in HS

4.3 Results

4.3.1 Study subjects

I recruited a new cohort of 15 patients with HS and age and sex matched controls. Mean age of patients was 39±13.2. There was a female predominance, in keeping with the gender distribution in HS. All had a raised BMI. Demographic details of the patients analysed for this study are outlined in table 4.1.

<table>
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<td>HS PATIENTS</td>
<td>39±13.2</td>
<td>13</td>
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<tr>
<td>HEALTHY CONTROLS</td>
<td>37±9</td>
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Table 4.1 Baseline characteristics of the study population
4.3.2 Gating strategy for MAIT cell identification in peripheral blood.

We first established a gating strategy for identification of MAIT cells in peripheral blood samples from hidradenitis suppurativa patients and controls (Figure 4.1). Lymphocytes were identified according to their Forward Scatter (FSC) and Side Scatter (SSC) properties, with doublet exclusion performed by plotting SSC-Area versus SSC-height. Next CD3 positive T cells were gated, after which TCR Va7.2, CD161 double positive cells were selected as MAIT cells.

Figure 4.1 Gating strategy for MAIT cell identification in PBMC
A. Single cell suspensions of PBMCs were analysed based on expression of CD45, followed by gating based on forward scatter (FSC) and side scatter (SSC). Representative scatter of CD45+ cells in PBMC samples one patient is shown. B. Singlets were then identified by SSC-area (SSC-A) versus SSC-height (SSC-H), followed by gating based on viability dye staining. C. T cells were identified based on the CD3+ population. D. MAIT cells were then identified as in the rest of the study, based on cd161 and Va7.2 co-expression.
4.3.3 T cells frequencies are the same in the blood of both healthy controls and HS patients

Before enumeration of MAIT cells was investigated, circulating T cell frequencies were measured in peripheral blood samples from hidradenitis suppurativa patients and controls. There was no difference in the circulating T lymphocytes between hidradenitis suppurativa patients and controls (Figure 4.2)

Figure 4.2 The percentage of T lymphocytes in healthy controls and HS patients.
Scatter plot displaying the frequencies of T lymphocytes (CD3+) in peripheral blood of H.S patients and controls. No significant difference noted.
4.3.4 MAIT cell frequencies are reduced in peripheral circulation of HS patients and display elevated PD-1 expression

We next determined MAIT cell frequencies and phenotype in hidradenitis suppurativa patients and controls. MAIT cells are presented as a percentage of the parent population as indicated and determined using flow minus-1 (FMO) and unstained controls. There was a reduction in the percentage of circulating MAIT cells in the HS group (HS 2.49% vs Healthy control 5.62%, p<0.01) in comparison to their healthy counterparts (Figure 4.3). We investigated the expression of the activation marker CD69 and the exhaustion marker PD-1 and noted no difference in CD69 expression but higher expression of PD-1 on MAIT cells from patients with hidradenitis suppurativa (Figure 4.3).

Figure 4.3 MAIT cell frequencies are reduced in peripheral circulation of HS patients. (A) Representative flow cytometric dot plot and scatter plots displaying the frequencies of MAIT cells (CD45+, CD3+, Va7.2+, CD161+) in peripheral blood of H.S patients or controls. (B) Scatter plot displaying the percentage expression of CD69 or PD-1 by MAIT cells from H.S patients or controls. Significant differences are indicated by *(p<0.05) and ***(p<0.001).
4.3.5 MAIT cells display altered cytokine profile in HS patients.

With the differences in MAIT cell frequencies and phenotype we next investigated the cytokine profiles of MAIT cells in peripheral blood samples from patients with hidradenitis suppurativa. MAIT cells were stimulated with TCR beads (anti-CD3/CD28) and IL-12/IL-18 for 18 hours, after which intracellular flow cytometry was used to investigate the production of IFNγ and IL-17. We observed increased production of IL-17 by MAIT cells from patients with hidradenitis suppurativa compared to controls (HS 7.1% vs Healthy control 1.39%, p<0.05). Patients with hidradenitis suppurativa also displayed reduced IFNγ when compared to healthy controls (Figure 4.4).

Figure 4.4 MAIT cells display an IL-17 phenotype in the peripheral blood of hidradenitis suppurativa patients.
A. Representative flow cytometric dot plot and scatter plots displaying the frequencies of MAIT cells stimulated with TCR microbeads/IL-18/IL-12 (50ng/ml) producing IL-17 in peripheral blood of H.S patients or controls. B. Scatter plots displaying the
4.3.6 Gating strategy for MAIT cell identification in infiltrating lymphocytes

With the observations of altered MAIT cell frequencies and phenotypes in the periphery of patients with hidradenitis suppurativa we moved our investigations into skin. We established a gating strategy for identification of MAIT cells in both lesional and adjacent skin from hidradenitis suppurativa patients (Figure 4.5). Skin resident CD45+ cells were gated with doublet exclusion performed by plotting SSC-Area versus SSC-height. Next CD3 positive T cells were gated, after which TCR Va7.2, CD161 double positive cells were selected as MAIT cells.

![Gating strategy for MAIT cell identification in skin](image)

**Figure 4.5 Gating strategy for MAIT cell identification in skin**

A. Single cell suspensions from lesional or adjacent skin were analysed based on expression of CD45, followed by gating based on forward scatter (FSC) and side scatter (SSC). Representative scatter of CD45+ cells in one patient is shown. B. Singlets were then identified by SSC-area (SSC-A) versus SSC-height (SSC-H), followed by gating based on viability dye staining. C. T cells were identified based on the CD3+ population. D. MAIT cells were then identified as in the rest of the study, based on cd161 and Va7.2 co-expression.
4.3.7 MAIT cells frequencies are increased in HS lesions

We next investigated MAIT cell frequencies in lesions and adjacent skin of patients with hidradenitis suppurativa. We observed increased MAIT cells frequencies in lesional skin compared to adjacent skin (HS lesion 4.25% vs adjacent skin 0.75%, p<0.001) (Figure 4.6).

**Figure 4.6 MAIT cells accumulate in lesions of hidradenitis suppurativa patients.**
Scatter plot displaying the frequencies of MAIT cells (CD45+, CD3+, Va7.2+, CD161+) in H.S lesions or adjacent skin from H.S patients. Significant differences are indicated by ***(p<0.001).
4.3.8 MAIT cells display altered cytokine profile in HS patients

Having demonstrated an accumulation of MAIT cells in the lesional skin of patients with hidradenitis suppurativa we next investigated the cytokine profile. Similar to peripheral blood experiments, MAIT cells were stimulated with TCR beads and IL-18/IL-12 for 18 hours. We show that MAIT cells in lesions produce significantly elevated levels of IL-17 compared to adjacent skin (HS lesion 21% vs Adjacent skin 4%, p<0.01) (Figure 4.7). In contrast to peripheral blood, no difference in IFNγ was noted (Figure 4.7).

**Figure 4.7 MAIT cells display an IL-17 phenotype in lesions of hidradenitis suppurativa patients.**
A. Representative flow cytometric dot plot and scatter plots displaying the frequencies of MAIT cells stimulated with TCR microbeads/IL-18/IL-12 (50ng/ml) producing IL-17 in lesions or adjacent skin of H.S patients. B. Scatter plots displaying the frequencies of MAIT cells stimulated with TCR microbeads/IL-18/IL-12 (50ng/ml) producing IFNγ
in lesions or adjacent skin of H.S patients. Significant differences are indicated by *(p<0.05), **(p<0.01) and ***(p<0.001).

4.3.9 Inhibition of RORγt in MAIT cells from HS patients results in reduced IL-17 production

With the observation of increased IL-17 production by MAIT cells from patients with hidradenitis suppurativa we next investigated the transcriptional regulation of IL-17. IL-17 expression is controlled by the transcription factor RORγt, so we investigated RORγt expression in MAIT cells from HS patients and demonstrate elevated RORγt expression in both peripheral and lesional MAIT cells compared to control MAIT cells (Figure 4.8A). We next investigated if targeting RORγt using a specific inhibitor could reduce IL-17 production by MAIT cells in vitro. Firstly, we generated MAIT cell lines which robustly produced IL-17 (Figure 4.8B). Treatment with RORγt inhibitor resulted in reduced IL-17 transcription and secretion (Figure 4.8C-D). To confirm in the setting of H.S we treated samples from HS patient and demonstrate a robust reduction in IL-17 production (Figure 4.8E).
Figure 4.8 Targeting RORγt in MAIT cells reduces IL-17 in hidradenitis suppurativa patients. A. Representative flow cytometric histogram and scatter plot displaying the expression of RORγt in MAIT cells from H.S patients (peripheral blood or lesion) or healthy controls (n=4). B. Scatter plots displaying the levels of IL-17 (pg/ml) produced by expanded primary MAIT cells alone or stimulated with TCR microbeads/IL-18/IL-12 (50ng/ml) for 18 hours. C-D. Scatter plots displaying the levels of IL-17 (pg/ml) or IL-17 mRNA expression in expanded primary stimulated MAIT cells with or without SR1001 (5µM) treatment. E. Scatter plot displaying the fold change in IL-17 in H.S patient cells treated with SR1001 (5µM) (n=6). Data is representative of minimum 5 independent experiments unless otherwise stated. Significant differences are indicated by *(p<0.05), **(p<0.01) and ****(p<0.001).
4.4 Discussion

MAIT cells are a population of innate T cells which are implicated in the pathogenesis of many inflammatory conditions including some skin diseases\textsuperscript{80-82}. They are capable of rapidly producing several cytokines, including TNF-alpha, IFN-gamma and IL-17\textsuperscript{111}, which have also been reported in HS\textsuperscript{2}. Several immune subsets have been studied in HS but the contribution of MAIT cells to the inflammatory burden has not yet been investigated. My overall aim was to investigate their presence in the disease, whether they play a role in HS pathogenesis and if so, do they represent a potential therapeutic target.

Firstly, we made the novel observation MAIT cells frequencies are reduced in the peripheral blood of patients with HS and accumulate in lesions but not in the adjacent skin (Figure 4.6). This is parallel to previous work in MAIT cells and skin disease; Teunissen et al found IL-17A-producing CD8\textsuperscript{D} MAIT cells were predominantly in psoriatic plaques and were almost absent in healthy skin\textsuperscript{80}. Li et al found the proportion of MAIT cells infiltrating the skin to be selectively increased in dermatitis herpetiformis, suggesting a role for MAIT cells in the pathogenesis of this disease\textsuperscript{93}. Again, similar to our observations, MAIT cells are noted to accumulate in the tissue of several other inflammatory diseases. Carolan et al investigated MAIT cells’ role in obesity and found they are enriched in human adipose tissue\textsuperscript{75}. In addition, MAIT cells have been shown to migrate into the synovial fluid in Rheumatoid Arthritis\textsuperscript{89} and ankylosing spondylitis\textsuperscript{90}. In Sjögren syndrome MAIT cells were found to infiltrate the salivary glands\textsuperscript{91} and are reported to be increased in the inflamed tissue in Inflammatory bowel disease (IBD)\textsuperscript{92}. 

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Secondly, to build on this finding of an accumulation of MAIT cells in the HS lesions, we wanted to determine if the MAIT cells were functionally different, so we investigated their cytokine profiles. We found MAIT cells display an altered cytokine profile in HS patients with a dominant IL-17 bias. There was an increased expression of the IL-17 producing MAIT cells in both the periphery and lesional skin of H.S patients when compared to healthy controls (Figure 4.7).

Increased IL17 producing MAIT cells are well documented in other conditions and have been highlighted as potential contributors to the pathogenesis of disease. In a murine model of SLE, IL-17 producing MAIT cells were expanded and their deletion resulted in ameliorated disease severity\textsuperscript{112}. Three separate studies that investigated MAIT cells in ankylosing spondylitis (AS), reported that IL-17 production by MAIT cells was higher, again suggesting that IL 17 MAIT cells play a role in the pathogenesis\textsuperscript{70}. These findings are further supported by a study in IBD which found that increased IL-17 producing MAIT cells levels in the inflamed bowel correlated with disease activity, suggesting their possible pathogenic role\textsuperscript{113}.

Moreover, there is increasing literature implicating IL-17 in the pathogenesis of HS. Kelly et al demonstrated that CD4\textsuperscript{+} T cells produce IL-17 in HS and suggested that the IL-17 pathway may be important in HS pathogenesis\textsuperscript{59}. In concordance with this, Lima et al reported IL-17-producing cells are present in lesional and perilesional HS skin and may contribute to the initiation of the inflammatory processes\textsuperscript{86}. In addition, another group reported significantly elevated IL-17 serum levels in patients with HS compared to controls\textsuperscript{60}. 
Encouragingly, given the accumulating evidence of a key role of the IL-17 pathway in HS pathogenesis, there has been considerable interest in the therapeutic targeting of IL-17 with positive results\textsuperscript{114,115}. The authors note, however, given the association between HS and Crohn’s disease, IL-17A inhibition in HS may be problematic and cases of IBD while on treatment have been reported\textsuperscript{116}.

Following on from observing an increased expression of IL-17 producing MAIT cells in both the periphery and lesional skin of H.S patients, we wanted to assess their expression of ROR\textsubscript{gt}. As mentioned, the Retinoic acid receptor-related-\gamma\textsubscript{t} (ROR\textsubscript{gt}) is the key transcription factor required for Th17 cell differentiation and for the production of the IL-17 cytokines by innate and adaptive immune cells\textsuperscript{83}. Not surprisingly we found an increased expression of ROR\textsubscript{gt} in MAIT cells in both the periphery and lesions of HS patients, compared to healthy controls and adjacent skin respectively. (Figure 4.8A)

To date the effect of ROR\textsubscript{gt} inhibition on MAIT cells has not been reported. Small molecular inhibitors of ROR\textsubscript{gt} were first described in 2011 by Huh and colleagues who demonstrated efficacy in murine models of autoimmune inflammation\textsuperscript{8}. Since then, several studies have investigated the targeted inhibition of ROR\textsubscript{gt} as an alternative to anti-IL-17 biological therapy\textsuperscript{9}. In spondylarthritis patients ROR\textsubscript{gt} inhibition selectively targeted IL-17 producing iNKT and \gamma\delta-T cells\textsuperscript{87}. Similarly, Guendisch and colleagues demonstrated positive effects of ROR\textsubscript{gt} inhibition in murine models of arthritis\textsuperscript{88}. To this end we assessed the potential impact of ROR\textsubscript{gt} inhibition on MAIT cells in primary MAIT cell lines and MAIT cells from H.S patients and showed significant inhibition of IL-17 (Figure 4.9).
These results highlight the potential use of small molecule RORγt inhibitors in the treatment of HS. Takaishi et al described how oral administration of A213, a novel RORγt antagonist, resulted in attenuation of skin inflammation in two independent psoriasis mouse models\textsuperscript{117}. Smith et al. reported that topical treatment with the RORγt inverse agonist GSK2981278 might improve clinical outcomes of psoriasis patients\textsuperscript{118}. From a translational aspect, these studies support the concept that RORγt inhibition can be achieved by the oral or topical route; which compared to monoclonal antibodies, involving injections and huge economic costs, would be preferable. In
addition, with the implication of IL-17 producing MAIT cells in numerous human chronic inflammatory conditions, these may provide a novel therapeutic avenue beyond H.S.

4.5 Conclusion

Collectively our data shows, for the first time, an accumulation of IL-17 producing MAIT cells in hidradenitis suppurativa lesions, and provides supporting data for investigating RORγt small molecule inhibitors in the treatment of patients with HS.

4.6 Key Findings

- MAIT cells accumulate in the lesions of HS patients
- Lesion microenvironment polarizes MAIT cells to an IL-17 phenotype
- The IL-17 transcription factor RORγt is elevated in MAIT cells from HS patients supporting the type-17 phenotype
- Targeting RORγt strongly reduces IL-17 production by MAIT cells and represents a potential therapeutic target
Chapter Five: Concluding discussion and future directions

My thesis reports two main studies which have HS as a central theme. To start, I established there is a high frequency of IR in our HS cohort and those patients were more likely to be obese.

My next study made the novel observation that there is an accumulation of IL-17 producing MAIT cells in hidradenitis suppurativa lesions. In addition, we demonstrated inhibition of RORγt in MAIT cells from HS patients results in reduced IL-17 production, supporting data for investigating RORγt small molecule inhibitors in the treatment of the condition. This represents an exciting avenue for future research given the limited range of evidence-based therapies and the absence of curative treatments\textsuperscript{15} for the condition.

Interestingly, there is emerging consensus that suggests that inflammation links obesity, metabolic dysfunction and inflammatory disorders\textsuperscript{50,51}, but whether there is a link between IR, HS and MAIT cells remains unknown.

As mentioned, several studies have confirmed that patients with HS have a higher body mass index (BMI) compared to the general population\textsuperscript{3} and that also reflects what I found in my study.

We know in obesity MAIT cells have been shown to have an impact on insulin resistance; Carolan et al found that in obese adults, there was an increase in the frequency of IL-17–producing MAIT cells and the change in MAIT cell frequency was
greater in obese adults with uncontrolled HbA1c. Their data suggests that changes in MAIT cell frequencies and functions may be contributing to insulin resistance and type 2 diabetes mellitus (T2DM)\textsuperscript{75}. Interestingly we too found increased numbers of IL17 producing MAIT cells in our HS cohort.

In addition, as I previously described, the proinflammatory cytokine IL-17 is now considered one of the major pathogenic players in HS and studies have reported it also has a key role in the impairment of insulin sensitivity. Zuniga et al found IL-17 inhibits adipogenesis, moderates adipose tissue (AT) accumulation, and regulates glucose metabolism in mice. Similarly, Fabbrini et al found IL-17 signature cytokines induce insulin resistance in skeletal muscle cells and hepatocytes\textsuperscript{119}. Although these studies suggest an intricate interaction between IL-17, IR and inflammatory disease, this requires further work. Future research investigating whether MAIT cell frequencies and function are altered in HS patients who are insulin resistant versus those with a normal HOMA-IR would be helpful in answering this question.

Additionally, this work has shown MAIT cells are accumulating in HS lesions and driven by ROR\textgamma, but further studies are required to investigate MAIT cell tissue homing, phenotype & trafficking i.e. how MAIT cells get to the HS lesion. Ultimately, murine models are required to give a clearer indication of the role MAIT cells are playing in HS pathogenesis.

In summary, this is the first study to report MAIT cells and their function in HS. I also demonstrated increased IR in our HS patients. MAIT cells are implicated in both disease processes, however, whether these are linked requires more investigation.
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Kelly, Natacha Veerapen, Gurdyal Besra, Ronan Bergin, Nicholas Jones, Donal O’She, Linda V. Sinclair, Andrew E. Hogan

Human Mucosal Associated Invariant T cell proliferation is dependent on a MYC-SLC7A5-Glycolysis metabolic axis

bioRxiv 2022.01.17.476571; doi: https://doi.org/10.1101/2022.01.17.476571


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related?" *Clinical and Experimental Dermatology*, vol. 34, no. 6, pp. 694–697, 2009.


Appendix A - Patient Information Leaflet

Department of Dermatology, ADELAIDE AND MEATH HOSPITAL, INCORPORATING THE NATIONAL CHILDREN’s Hospital

PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE:
Innate immune regulation of skin disease: Hidradenitis suppurativa

NAME OF PRINCIPAL INVESTIGATORS:
Dr Anne – Marie Tobin

You are being invited to participate in a research study. Thank you for taking time to read this.

WHAT IS THE PURPOSE OF THIS STUDY?
This study aims to see if certain immune cells known as innate immune cells are changed in the skin or blood of patients with hidradenitis suppurativa.

WHY HAVE I BEEN CHOSEN?
You have been chosen because you have hidradenitis suppurativa.

WHAT WILL HAPPEN IF I VOLUNTEER?
Your participation is entirely voluntary. If you decide to take part you can change your mind at any time. This will not affect your future treatment in any way. Furthermore, your doctor may decide to withdraw you from this study if he/she feels it is in your best interest.

If you wish to take part we will take a blood samples to measure certain mediators of metabolism and inflammation. This will involve around 4 tablespoonfuls of blood.

A skin biopsy will also be taken from an area of hidradenitis suppurativa and from skin which does not have hidradenitis suppurativa. This involves the injection of a small amount of local anaesthetic which is painful, it feels like a bee-sting. Once numb a
small round piece of skin around 5 mm will be taken, and one to two dissolvable stitches inserted.

ARE THERE ANY RISKS INVOLVED IN PARTICIPATING?
Taking blood samples may cause a bruise and some pain. A skin biopsy is painful, the local anaesthetic feels like a bee-sting, it may also leave a small scar. About 1 in 100 patients who have a skin biopsy may get an infection in the surrounding skin.

WHAT HAPPENS IF I DO NOT AGREE TO PARTICIPATE?
If you decide not to participate in this study you are free to withdraw at any time and your treatment will not be affected in any way.

CONFIDENTIALITY
Any information collected during the study will remain confidential and will not be disclosed to anyone outside the research team. Results obtained from the study may be published in scientific journals, oral and poster presentations but your identity will remain anonymous.

COMPENSATION
Your doctors are insured by the State Claims Insurance Service.

WHO IS ORGANISING AND FUNDING THIS RESEARCH?
This study is organised and funded by the Department of Dermatology at Tallaght Hospital, Tallaght, Dublin 24.

HAS THIS STUDY REVIEWED BY AN ETHICS COMMITTEE?
The St James and Tallaght Hospital Ethics Committee has approved this study.

CONTACT DETAILS
Name: Dr Anne – Marie Tobin,  
Address: Consultant Dermatologist  
Tallaght Hospital,  
Tallaght,  
Dublin 24.  
Phone: 01-4142105
Appendix B: Consent form

Innate immune regulation of skin disease: Hidradenitis suppurativa

PLEASE TICK YOUR RESPONSE IN THE APPROPRIATE BOX

- I have read and understood the Participant Information      YES  NO
- I have had the opportunity to ask questions and discuss the study   YES  NO
- I have received satisfactory answers to all my questions   YES  NO
- I have received enough information about this study   YES  NO
- I understand that I am free to withdraw from the study at any time without giving a reason and without this affecting my future medical care      YES  NO
- I agree to take part in the study   YES  NO

Participant’s Signature: ____________________________  Date: _________
Appendix C - Data Collection Proforma

**INNATE IMMUNE REGULATION OF SKIN DISEASE**

**HIDRADENITIS SUPPURATIVA**

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HS SYMPTOMS DURATION: ________ MONTHS

HS DIAGNOSIS: ________ MONTHS

HURLEY STAGE:

IS THERE A FAMILY HISTORY OF HS? YES / NO

PREVIOUS TREATMENT

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PREVIOUS SURGERY? YES/NO

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HISTORY OF PCOS : YES / NO

HISTORY OF DM : YES / NO

SMOKING HISTORY: ___ /DAY  ___ YEARS

C2H5OH: _____ /WEEK