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**Pathophysiology of Pruritus in
Cutaneous T-cell Lymphoma, Mastocytosis and Psoriasis *vulgaris***

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Unanswered questions make my head itch.

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Abstract

Chronic pruritus is a major and distressing symptom common to several conditions that arises from a complex dialogue between skin cells and neurons, sustained by mediators, such as cytokines, neurotransmitters, endothelial adhesion molecules, angiogenic factors and other biomolecules. Pruritus is a common symptom in cutaneous T cell lymphoma (CTCL), mastocytosis and psoriasis. The response to similar therapies is often different between the diseases and between different clinical stages of the same condition, strengthening the need to clarify the underlying pathways, cells and mediators involved in pruritus in these diseases.

The goal of this study was to clarify the mechanisms underlying pruritus in CTCL, mastocytosis and psoriasis patients. For that, we measured the levels of different cytokines (IL-8, IL-31 and IL-33), neuromediators (serotonin), endothelial adhesion molecules (E-selectin) and angiogenic factors (vascular endothelial growth factor; VEGF) in these patients. For each disease under study, the correlation of the several biomarkers with itching score and severity of the disease was studied.

We found that CTCL patients presented increased levels of IL-31 and E-selectin, especially patients with Sezary Syndrome (SS); moreover, E-selectin correlated with pruritus in SS. In Mycosis Fungoides (MF) serotonin presented a tendency for higher levels. Pruritus correlated with tryptase levels in mastocytosis. Finally, although we found elevated levels of IL-8, E-selectin, VEGF and C-reactive protein (CRP) in psoriasis, they did not correlate with pruritus.

In summary, our data suggest that serotonin and tryptase are important biomarkers in mastocytosis; the high levels of E-selectin and CRP in the severer forms of psoriasis suggest that these parameters are potential biomarkers of psoriasis severity; in patients with SS, E-selectin and IL-31 appear to be important in itchy pathways; in MF patients the increase in serotonin seems to be more important in the mechanisms underlying pruritus. However, more studies are needed to clarify itch mechanisms in the three diseases.

Our data strengthens the hypothesis that different mechanisms underlie the genesis of itch, explaining the different therapeutic responses to common treatments, and proposes some potential therapeutic targets for the treatment of itch in CTCL, mastocytosis and psoriasis patients.

Key-words: Pruritus; Cutaneous T-cell Lymphoma; Mastocytosis; Psoriasis; Inflammation

Resumo

O prurido crônico é um sintoma importante e comum a várias doenças, que resulta de um diálogo complexo entre células da pele e neurônios, sustentado por mediadores, como citocinas, neurotransmissores, moléculas de adesão vascular, fatores angiogênicos e outras biomoléculas. O prurido é um sintoma comum no linfoma cutâneo de células T (CTCL), na mastocitose e na psoríase. A resposta a terapias similares frequentemente difere entre as várias doenças e entre diferentes estádios clínicos da mesma doença, reforçando a necessidade de esclarecer quais as vias de sinalização, células e mediadores subjacentes ao prurido nestas doenças.

O objetivo deste estudo foi clarificar os mecanismos envolvidos na gênese do prurido no CTCL, na mastocitose e na psoríase. Para isso, medimos os níveis de diferentes citocinas (IL-8, IL-31 e IL-33), neurotransmissores (serotonina), moléculas de adesão vascular (E-seletina) e fatores angiogênicos (fator de crescimento endotelial; VEGF), nestes doentes. Para cada doença, as correlações entre os diferentes biomarcadores com o nível de prurido e a severidade da doença foram estudadas.

Os nossos resultados mostram que os doentes com CTCL apresentam níveis aumentados de IL-31 e E-seletina, particularmente os doentes com síndrome de Sezary; para além disso, neste doentes, os níveis de E-seletina correlacionaram-se com o prurido. Na mycosis fungoides, a serotonina apresentou valores tendencialmente mais elevados. Na mastocitose, o prurido relacionou-se com os níveis de triptase. Finalmente, embora tenhamos encontrado níveis aumentados de IL-8, E-seletina, VEGF e proteína C-reativa (CRP) nos doentes de psoríase, estes não se relacionaram com o prurido.

Em suma, os nossos resultados sugerem que a serotonina e a triptase são importantes biomarcadores na mastocitose; os níveis elevados de E-seletina e de CRP, nas formas mais severas de psoríase, sugerem que estes parâmetros são potenciais biomarcadores da severidade desta doença; nos doentes com síndrome de Sezary, a E-seletina e a IL-31 parecem ser importantes nas vias pruriceptivas; nos doentes com mycosis fungoides, a serotonina parece ser um importante biomarcador. Contudo, são necessários mais estudos para identificar os mecanismos envolvidos no prurido destas três doenças.

Os nossos dados suportam a hipótese de que diferentes mecanismos participam na gênese do prurido, explicando as diferentes respostas à terapêutica, e propõem potenciais alvos terapêuticos para o tratamento do prurido nos doentes com CTCL, mastocitose e psoríase.

Palavras-chave: Prurido, Linfoma Cutâneo de células T, Mastocitose; Psoríase; Inflamação

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Abbreviations

APC – Antigen Presenting Cell	QoL – Quality of Life
CHP – Centro Hospitalar do Porto	SCF – Stem Cell Factor
CHUC – Centro Hospitalar da Universidade de Coimbra	SM – Systemic Mastocytosis
CM – Cutaneous Mastocytosis	SP – Substance P
CMiHis+ – Mechano-insensitive Histamine-positive C-units	SS – Sezary Syndrome
CRP – C-reactive protein	STT – Spinothalamic Tract
CTCL – Cutaneous T-cell Lymphoma	Th – T-helper
DLQI - Dermatology Life Quality Index	TNF – Tumour Necrosis Factor
ELAM-1 – Endothelial-leukocyte adhesion molecule 1	TNMB – Tumour-Node-Metastasis- Blood
ELISA – Enzyme Linked Immunosorbent Assay	UP – Urticaria Pigmentosa
FNE – Free Nerve Endings	VAS – Visual Analogue Scale
HIV – Human Immunodeficiency Virus	VEGF – Vascular Endothelial Growth Factor
HLA – Human Leukocyte Antigen	WBC – White Blood Cell
IFN– Interferon	
IFSI - International Forum for the Study of Itch	
Ig - Immunoglobulin	
IL – Interleukin	
IQR – Interquartile range	
KIT – Transmembrane receptor tyrosine kinase (CD117)	
MC – Mast Cell	
MF – Mycosis Fungoides	
PASI – Psoriasis Area and Severity Index	
PRO – Patient-related Outcomes	
PruNet – European Network on Assessment of Severity and Burden of Pruritus	
PV – Polycythaemia Vera	

Introduction

Chronic pruritus is a major and distressing symptom common to several conditions that can significantly impair the patients' health-related quality of life (QoL). One of the first scientists that studied itch was von Frey, proposing an intensity hypothesis for itch and claiming that itch was a milder form of pain. Years later, Lewis reported that pain had two presentations: the "first pain", pricking, sharp, superficial and localized; and the "second pain", the burning pain, more diffuse and deep, following the first pain (1). Similarly, Graham *et al.* also reported that itch caused by the plant spicule cowhage (the itch powder) had two components mediated by different nerve fibres: the pricking itch was mediated by myelinated A- δ fibres that lead to the first pain, and the burning itch was mediated by unmyelinated C-fibres that lead to the second pain (2). They also found that itchy skin had a lower threshold for pain, that itch could be abolished by painful stimuli, and that areas of hyperalgesia were insensitive to itch. Pain and itch thus appeared to be processed by the same neuronal circuits (1); nowadays, it is known that both pain and itch are transmitted by the anterolateral system (3).

The pattern theory, one of the two most reliable theories concerning pruriceptive sensory processing in the nervous system, postulates that stimuli activate different kinds of nociceptors and spinal cord neurons and that the sum of the neuronal activity determines the sensation felt. According to this theory, the *pattern* of activation determines that itch is felt upon pruriceptive stimuli, and not pain or non-noxious touch (4). However, evidences suggest a distinct neuronal pathway for pain and itch: for instance, certain kinds of pain don't turn into itch when the stimuli get weaker; itch can be very intense and bothersome without turning into pain; opioids decrease pain but increase itch and while pain elicits a withdrawal behaviour, itch leads to scratching. Pain can be felt in virtually all the body, from the surface to muscles and inner organs, while itch can only be felt in the skin and mucosal surfaces (5). Neuroimaging studies revealed that different areas of the brain are activated during itch and pain (6). These observations are in agreement with the second and most accepted theory for itch: the specificity theory, that claims a *specific* pathway of neurons along the central and peripheral nervous systems, responsible for the translation and transmission of pruriceptive information (4).

1 Neuroanatomy and physiology of itch

Itch is somatosensory, as pain, temperature, and others. Sensory stimuli are processed by the sensory system, which has an ascending (afferent) pathway in the nervous system. Itch perception begins in skin receptors and mucosal surfaces. This sensory information is converted to neuronal signals that travel through the spinal cord into higher areas of the brain, namely the thalamus and the somatic sensory cortex. The system of neurons that transmit itch, pain, temperature and, in a less extension, touch, is the anterolateral or spinothalamic system (3, 7).

1.1 Primary afferent fibres

Pruritogens activate the free (or bare) nerve endings (which comprise the sensory portion) of primary afferent fibres, also called dorsal root ganglion neurons (3). The branches of dorsal root ganglion neurons collect in the dorsal root.

Free nerve endings (FNE) of primary afferent fibres have membrane receptor channel complexes that convert the received stimuli into electric signals. These membrane receptor channels are different, regarding the type of substances to which that fibre is sensitive (8). FNE of C-fibres and A- δ fibres subserve different sensory modalities (9). The most common type of C-fibres, the polymodal C-fibres, respond to painful, mechanical and heat stimuli, but they are not, or are very weakly, activated by histamine. Histamine-responsive afferents are a subtype of unmyelinated C-fibres that possess wide enervation territories, are mechanically unresponsive and have slow conduction velocities (10, 11), termed mechano-insensitive histamine-positive C-units (CMiHis+). Although considered itch-specific, these afferent fibres are responsive to heat (10), as well as to some algogens (substances that cause pain) (11). This response to noxious stimuli of CMiHis+ units may explain the abolishment of itch by pain and, thus, the scratching reflex in an itchy area (11).

Cowhage also triggers itch by activating mechanically sensitive C-fibres (4), indicating that itch is mediated by more than one type of C-afferent fibres. Additionally, myelinated A- δ fibres seem to have an important role in non-histaminergic cowhage-mediated itch (12).

1.2 Spinal cord

Electrical signals flow through the axons into the cell bodies of primary afferent fibres, synapsing on second-order neurons located in the dorsal column nucleus of the dorsal horn of the spinal cord (4). Pruriception ascends the brain from the spinal cord via the spinothalamic tract (STT). Although stimuli caused by both histamine and cowhage activate STT neurons, they never activate the same cell (13), indicating that cowhage and histamine

activate different types of afferent fibres and of STT neurons, despite sharing a common pathway, supporting the hypothesis of more than one mechanism for itch.

1.3 Brain

The axons of dorsal column nucleus neurons reach the contralateral thalamus. From here, axons project to the somatic sensory cortex of the parietal lobe in the cerebral hemispheres (3).

1.4 Pain and itch inhibition in the nervous system

The STT has two projections, one of which is to the periaqueductal grey matter that surrounds the cerebral aqueduct in the midbrain. This is a key region in pain modulation, as it is from here that arises a descending pain inhibitory system. The periaqueductal grey matter receives emotional information from higher structures of the brain. Then, excitatory neurons project to nuclei in the medulla and then the dorsal horn of the spinal cord, using serotonin to directly inhibit ascending neurons that transmit painful information and activate inhibitory interneurons (3).

Pain seems to act as a counter-stimulus for itch, as scratching relieves this symptom. Studies have proven that painful heat, scratching (14, 15) and cold (16) relieved histaminergic itch, the latter being mediated by the activation of A- δ fibres.

2 Mediators of itch

Pruriceptive information is translated and conducted by molecular signals via inflammatory mediators, neurotransmitters and both endogenous and exogenous substances. Pruritogens activate receptors in FNE, exciting neurons to release neurotransmitters to communicate the information to next-level neurons in the STT.

2.1 Amines

Histamine has been the most studied pruritogen and is considered the classic itch mediator. Like serotonin, histamine is a biogenic amine. After intradermal injection or intrinsic release of histamine from activated mast cells and basophils, a local reaction occurs. Histamine causes vasodilation of local arterioles and an erythema spreads several centimetres around the area where it occurs, originating a flare. Vasodilation also allows plasma extravasation from the blood vessels, with subsequent oedema or wheal formation in the skin (17). This sequence of events (flare, spread of the flare and wheal) is known as the “triple response”. Histaminergic itch is triggered by stimulation of histamine receptors in FNE. A molecular signalling pathway results in the depolarization of the nerve fibres via activation of voltage-dependent sodium channels (18).

Serotonin is a neurotransmitter synthesized from the aminoacid tryptophan. Intradermal injection of serotonin causes itch, although to a less extent than histamine (11). Serotonin-mediated itch is processed by the same molecular signalling cascade as histamine (18), although it appears to be independent of it. In fact, serotonin induced non-histaminergic itch in the lesioned skin of atopic dermatitis patients (19) and an antagonist of serotonin receptor did not relieve pruritus induced by histamine in healthy subjects (20). A study reported that serotonin levels were increased in patients with mastocytosis, although they did not correlate with pruritus (21). Serotonin reuptake inhibitors improved the condition of patients with malignant disease (22) and also with psoriasis (23). It is believed that serotonin is also implicated in the pruritus of cutaneous T-cell lymphoma (CTCL) (1).

2.2 Neuropeptides

Substance P (SP) is a neurotransmitter highly important in the communication between primary afferents and the central nervous system. SP is released upon activation of FNE, which induce mast cells to release histamine with the subsequent triple response and itch (24, 25). Degranulated mast cells also release other inflammatory mediators, such as prostaglandins, leukotrienes (26) and cytokines (27), leading to a further release of SP by FNE and activation of mast cells (26, 28), keratinocytes and endothelial immune cells (8, 28). It thus appears that SP is not a pruritogen by itself, but, instead, acts as a mediator for

histaminergic itch. SP has also a role in inflammatory and systemic disease, as experiments with a SP receptor antagonist relieved itch in patients with chronic pruritus of various origins (29) and in patients with pruritus due to CTCL unresponsive to other treatments (30).

2.3 Cytokines

Cytokines are immunomodulators released mainly by leukocytes and some of them seem to have an important role in pruritus in different conditions. Interleukin (IL)-2, IL-31 and IL-33 have been associated with pruritus in some diseases, such as CTCL, mastocytosis and psoriasis.

IL-2, a cytokine involved in the proliferation and maturation of T-cells, causes itch in healthy subjects and atopic dermatitis patients (31); the dermis of psoriatic patients with pruritus presented increased levels of IL-2, compared to non-pruritic psoriatic skin (32); plasma levels of IL-2 were also increased in haemodialysis patients with uremic pruritus (33); an IL-2 antagonist relieved pruritus in a Sezary syndrome case study (34).

IL-8 is produced by antigen presenting cells (APCs), keratinocytes, mast cells and other cell types. This chemotactic cytokine, known to be increased in psoriasis and to correlate with its severity (35), induces neutrophil mobilization, transmigration and degranulation. IL-8 mRNA expression may be enhanced by histamine and tumour necrosis factor (TNF)- α via stimulation of histamine-4 receptors in keratinocytes, which are involved in pruritus and skin inflammation (36). IL-8 is also involved in the pathogenesis of malignancies, such as mastocytosis (37) and CTCL (38). However, Vaa *et al.* did not find a correlation between IL-8 levels and pruritus in primary myelofibrosis patients (39).

IL-31 is a cytokine synthesized by a variety of cells, including lymphocytes and mast cells (40) that has been proposed as a potential mediator in the genesis of pruritus in several diseases. Increased levels of IL-31 have been found in dermatologic diseases, such as atopic dermatitis (41), as well as in proliferative disorders like mastocytosis (42), CTCL (43) and polycythaemia vera (PV) (44). Although some data showed that IL-31 correlates with pruritus in CTCL (45, 46), other reported otherwise (47). Furthermore, IL-31 receptors have been reported in dorsal root ganglion neurons, indicating that IL-31 may directly activate sensory neurons (41) and cause itch, without further interference of mediators.

IL-33, known to induce Th2 cytokine responses, seems to be involved in association with IL-31 in the pathogenesis of skin side effects, including itch, observed in a patient treated with epidermal growth factor receptor tyrosine kinase inhibitors (48).

2.4 Arachidonic acid metabolites

Prostaglandins and leukotrienes are arachidonic acid metabolites synthesized via the cyclooxygenase and lipoxygenase pathways, respectively. These molecules seem to mediate itch rather than being direct pruritogens. In a study with healthy individuals, prostaglandin E2 enhanced histamine-induced pruritus (49). Leukotriene B4 concentration was increased in the affected skin of patients with atopic dermatitis and psoriasis (50).

2.5 Other mediators

E-selectin (also termed endothelial-leukocyte adhesion molecule 1; ELAM-1) is an important adhesion molecule of the inflammatory process, recruiting leukocytes into the site of injury. It was demonstrated that homing of T lymphocytes from Sezary syndrome patients in dermal microvessels in mouse ear skin was mediated by E-selectin and other adhesion molecules (51). Also, E-selectin had a two-fold increase in psoriatic skin with pruritus compared to non-pruritic lesioned skin (32). E-selectin also appears to be important in the process of angiogenesis (52).

Vascular endothelial growth factor (VEGF) is a pro-angiogenic molecule that has been implicated in the pathogenesis of inflammatory and systemic diseases. VEGF is increased in psoriasis patients (35), is produced by mast cell infiltrates in the bone marrow of mastocytosis patients (53) and by malignant CTCL cells (54). VEGF seems to have a particular role in pruritus, as increased levels correlated with disease activity in prurigo simplex, a chronic inflammatory skin condition; moreover, pruritus improved in a patient after treatment with bevacizumab, a monoclonal VEGF antibody (55).

2.6 Mucunain

Known as itch powder, cowhage are the spicules of the plant *Mucuna pruriens*. Cowhage invokes itch of higher intensity than that produced by electric skin stimulation or intradermal injection of histamine and is mediated by the protease mucunain (56). As mentioned before, histamine and cowhage activate different C primary afferents and STT neurons (13), what clearly defines separate pathways for itch perception.

3 Diseases associated with itch

Pruritus is classified as acute, when the patient complains of itch for less than six weeks, or as chronic pruritus, a debilitating symptom that accompanies many dermatological and systemic diseases. The International Forum for the Study of Itch (IFSI) proposed in 2007 a clinical and aetiological classifications for itch (57). For clinical purposes, group I is defined as pruritus on primary inflamed skin, including dermatological disorders like psoriasis or atopic dermatitis; group II - pruritus on primary non-diseased, non-inflamed skin disorders, including systemic, neurological or psychiatric diseases in which pruritus is present but skin lesions are absent. However, there are systemic disorders that present with skin lesions and, on the other hand, there are also dermatological diseases in which the skin appears healthy. Therefore, a close examination by a dermatologist is of significant importance in all cases of pruritus. Finally, group III includes diseases that present skin changes, due to intense scratching, frequently observed in patients with chronic itch for several years. The underlying diseases in which pruritus is a symptom are also separated by categories - Category I: dermatological diseases; Category II: Systemic diseases, including diseases of pregnancy and drug-induced pruritus; Category III: Neurological; Category IV: Psychiatric/psychosomatic diseases; Category V: Mixed (pruritus that is due to more than one disease); and, Category VI: Other (no underlying cause can be found).

3.1 Dermatological diseases

Chronic, non-infectious skin conditions such as atopic dermatitis, eczema or psoriasis, are non-curable diseases that have implications in the patients' QoL and self-esteem. These dermatologic diseases are often associated with pruritus (58), being itch one of the most reported symptom in dermatology consults.

3.1.1 Psoriasis

Psoriasis is an inflammatory chronic recurrent skin condition that affects approximately 2% of the global population, accounting for nearly 125 million people worldwide (59). The word "psoriasis" derives from the Greek word *psora*, which means "itch". Psoriasis is identified by its clinical and histological features and is known to affect patients self-esteem and QoL, leading to professional and personal limitations (59). Also, most psoriasis patients (67-92%) have complains of pruritus, sometimes occasional, other times moderate to severe and often associated with depression (60, 61).

Psoriasis, characterized by abnormal proliferation of keratinocytes and vascular alterations, is triggered by genetic and non-genetic factors, such as infection, inflammation, stress, skin injury and drugs (62). Sun exposure appears to influence the geographic

distribution of this disease, as the prevalence of psoriasis increases with distance from the equator (59, 63). Populations from the Arctic appear to have a prevalence of psoriasis up to 12%, whereas it is practically non-existent in South America and Asia. Incidence appears to be higher in Europe than in the United States and increases with age (63). Children are less affected than adults. Psoriasis is classified according to the onset of the disease, as type I (early-onset) psoriasis, when the first peak occurs before the age of 40 (75% of all cases), and as type II psoriasis (late-onset), with the first manifestation occurring between 50-60 years of age (59, 63). The disease occurs equally in both genders.

Several genes have been implicated in the predisposition to this skin disease (59, 64). Psoriasis has been associated with certain human leukocyte antigen (HLA)-types such as HLA-Cw6, HLA-B13, HLA-B17, HLABw57, HLA-DR4 and HLA-DR7 (62), and those with HLA-Cw6 seem to have a 10-fold higher risk to develop a more severe disease (65).

3.1.1.1 Pathogenesis

Antigenic stimulation of skin immune cells, namely Langerhans cells, dendritic cells and other APCs, triggers an immune response with production of antimicrobial peptides and cytokines. Actually, APCs are capable of activating other immune cells, resulting in the recruitment of T-cells, neutrophils, mast cells and other inflammatory cells to the affected area (66, 67), through the release and expression of TNF- α , IL-8 and other inflammatory cytokines, and the expression of ligands to E-selectin and other adhesion molecules (59). If the epithelial barrier is lesioned, activated keratinocytes will synthesize structural and barrier proteins, in order to repair the injury and regain homeostatic balance. APCs communicate with T-cells, activating them to produce cytokines and other inflammatory mediators. Langerhans cells are also capable of travelling to lymph nodes, further stimulating more T-lymphocytes (66). Dermal T-cells are CD4⁺ T-helper (Th) cells and epidermal lymphocytes are CD8⁺ cytotoxic T cells (59). Dermal Th cells acquire an inflammatory phenotype, with clonal expansion of Th1/Th17 cells, leading to an enhanced production of interferon-gamma (IFN- γ), TNF- α , IL-1, IL-2, IL-6, IL-12, IL-17, IL-22 and IL-23 (59, 66). This cytokine pool allows for macrophage and dendritic cells-mediated keratinocyte proliferation and inflammatory cell infiltration seen in histologic samples of lesioned psoriatic skin, sustaining the whole process.

Mast cells produce, among others, VEGF and IL-8, increasing the permeability of surrounding blood vessels and helping in the recruitment of innate and adaptive immune cells (68). Neutrophils are a major player in the pathogenesis of psoriasis, helping in the recruitment and activation of T-cells and in the proliferation of keratinocytes (69). Keratinocytes produce chemotactic mediators, such as IL-6 and IL-8 that increase the

immune infiltrate in the skin (70), and VEGF, which leads to the increased vascularization of the tissue (71).

This milieu of inflammatory cytokines is not localized only in lesioned skin. In fact, psoriasis patients have increased circulating levels of inflammatory cytokines (35). This chronic immunoinflammatory process contributes to the higher predisposition of psoriasis patients to co-morbidities, such as cardiovascular disease (72), metabolic disorders, gastrointestinal disease, diseases of the nervous system and cancer (59).

3.1.1.2 Clinical manifestations

Psoriasis can present in various clinical forms, according to the type and localization of lesions. The most common clinical form of psoriasis is chronic plaque psoriasis or psoriasis *vulgaris*. It presents with red plaques (due to intense local neovascularization) with silver or white scales (due to keratinocyte hyperplasia) that are absent in non-lesioned skin (59).

3.1.1.3 Severity and Treatment

The severity of psoriasis is often evaluated by the Psoriasis Area and Severity Index (PASI) score, which is performed by the physician. It combines the evaluation of four body areas: head and neck, upper limbs, trunk and lower limbs. To assess affected body surface area, the proportion of skin affected in each area is given by a numerical score. Within each area, the severity of the lesions is measured by three signs, erythema, thickness/induration, and desquamation/scaling (73). A PASI score below 10 defines psoriasis as mild, between 10 and 20 as moderate, and above 20 as severe.

Given the clinical presentation and the associated co-morbidities, psoriatic patients have a significant impairment in their QoL; complaints of stigmatization, depression and low self-esteem are common; periods of higher stress (74) are usually linked to exacerbation of skin lesions, further contributing to diminish the self-esteem of the patients (59).

The Dermatology Life Quality Index (DLQI) is a 10-items questionnaire, aimed to evaluate the QoL related with skin diseases. Its subscales are: symptoms and feelings, daily activities, leisure, work and school, personal relationships and treatment (59). The total DLQI score is calculated by summing the scores of the 10 questions; the maximum score is 30 and the minimum is 0. The DLQI score banding proposes: 0-1 means "no effect on QoL"; 2-5, "small effect"; 6-10, "moderate effect"; 11-20, "very large effect"; 21-30, "extremely large effect". A total DLQI higher than 10 represents a skin disease with a very large effect on patients' QoL. Psoriasis patients with enhanced PASI values present, usually, higher DLQI scores (75, 76).

The choice of psoriasis therapy depends on several factors, such as disease severity, skin type, response to previous treatments, effect on patient QoL, and clinical history. Topical therapy is used for milder or limited forms of psoriasis, and may be used in combination with other therapies. Phototherapy, photochemotherapy, systemic and biological agents are used for moderate and severe forms of psoriasis. Systemic therapy includes drugs, such as methotrexate, cyclosporine and systemic retinoids (59). Biological drugs comprises TNF inhibitors (etanercept, adalimumab, infliximab), ustekinumab, that targets the p40 subunit of IL-12 and IL-23, and the agents that target IL-17 (e.g. secukinumab and ixekizumab).

3.1.1.4 Itch in Psoriasis

Pruritus is a common symptom in psoriasis patients; 70-98% of psoriasis patients suffer from mild to severe itch (60, 61, 77-80). It is not consensual whether pruritus correlates with the PASI score: some studies report a significant positive correlation (75, 79, 81, 82), in opposition to others (32, 60, 77, 78, 80). Stress enhances pruritus (77, 79, 82), further aggravating QoL in psoriasis patients.

Although the mechanisms involved in pruritus in psoriasis are not well understood, it is believed that the increase in nerve endings, induced by nerve growth factors, and the associated inflammation induced by neuropeptides, may decrease the threshold for sensibility to pruritic stimuli in affected skin (32, 79, 83). Some studies have showed that pruritus is not only felt in lesioned skin, but also in apparently healthy skin areas (60, 79, 81), suggesting a systemic mechanism for itch in psoriasis, in addition to a local pathway. It was reported that IL-2 levels were increased in pruritic psoriasis lesions, compared to non-pruritic lesioned skin, although IL-8 levels were similar (32). Concerning the possible role of IL-31 in psoriatic pruritus, some authors reported increased levels in psoriasis patients (84), while others reported otherwise (85). Sonkoly and colleagues found no differences in IL-31 levels between pruritic and non-pruritic psoriatic skin (41), but Narbutt *et al.* observed that both IL-31 and pruritus decreased after treatment with narrow-band ultraviolet light B (84); this reduction in pruritus, however, may be due to skin improvement and not IL-31 decrease *per se*. Indeed, Szepietowski *et al.* reported that itch diminished when skin lesions were cleared (81). Other mediators such as serotonin (23) and E-selectin (32) may also have a role in pruritus associated with psoriasis.

There is no specific treatment for pruritus in psoriasis. Usually, pruritus diminishes when skin condition improves.

3.2 Haematological malignancies

Pruritus has been associated with haematological disorders for decades, and is one of the symptoms that raises suspicion for malignancy when cutaneous lesions are absent (86). The term “paraneoplastic itch” is often employed when pruritus occurs in cancer patients; it is a rare symptom in most types of cancer, however it is frequently found in patients with blood malignancies (87), namely in PV, Hodgkin’s lymphoma, CTCL, mastocytosis, and others.

3.2.1 Cutaneous T-Cell Lymphoma (CTCL)

Primary cutaneous lymphomas are a rare and heterogeneous group of non-Hodgkin’s lymphomas, in which antigenic-stimulated malignant B and T lymphocytes with skin tropism proliferate and cause skin lesions.

CTCL is the most common form of primary cutaneous lymphomas. The majority of CTCL are malignant diseases of mature Th cells, including the variants Mycosis Fungoides (MF) and Sezary Syndrome (SS). MF/SS have an indolent course and are epidermotrophic disorders (88), often mimicking benign skin conditions, such as psoriasis or eczema (89). In fact, initial skin lesions are both clinically and histologically non-specific. Years or even decades may pass between appearance of skin lesions and the final and correct diagnosis (88, 90). MF is a milder form of the disease that, as it progresses, the malignant clone in both skin and circulation increases, leading to severe skin involvement and to secondary complications observed in SS patients. The indolent variants of CTCL progress from clinically and histologically non-specific skin lesions (premycotic) to an erythematous or eczematoïd stage, which may be accompanied by severe itching. The next stage, the infiltrative plaque stage, is characterized by indurated discrete papules, oval or round in shape. The tumour stage presents with ulcerative mass lesions in both previously injured and healthy skin (88).

Classic MF progresses slowly and presents with well-defined pruritic erythematous skin patches in sun-protected areas. These lesions may evolve to infiltrative plaques and tumours. Histologically, the skin presents with a band-like lymphocytic infiltrate, variable number of inflammatory cells, clusters of malignant cells and, occasionally, apoptotic keratinocytes. Tumour lesions have deeper dermal infiltration and diminished epidermotropism. Folliculotropic MF is the most common MF subtype, presenting with severe pruritus, skin plaques, cysts and lesions; alopecia and scarring may occur. Pagetoid reticulosis is an indolent form of MF with localized scaling patches on the extremities. Skin histology reveals prominent epidermal hyperplasia. Granulomatous MF presents a more rapid disease progression and poor prognosis. Clinically, it resembles classic MF and may

present with different histopathologic patterns (89). MF tumour cells may undergo transformation, from small atypical lymphocytes to a large cell variant (89). These large atypical cells are also observed in Pagetoid reticulosis and are positive for CD30 marker (91).

MF and SS may also present as erythrodermic CTCL, with mild and generalized exfoliative erythroderma, which causes electrolyte imbalances, hypothermia, eyelid changes and alopecia. These erythrodermic CTCL often resemble other dermatologic diseases, such as infections, psoriasis or eczema, and are usually considered as a progression of the disease without blood involvement (89). SS, the leukemic form of CTCL, is more aggressive than MF, presenting Sezary cells, atypical, skin-homing malignant CD4⁺ lymphocytes, with a highly convoluted nucleus, condensed heterochromatin at the periphery and abnormal cytogenetics, in the skin and peripheral blood (88). Clinically, besides erythroderma, SS presents severe and disabling pruritus and, in some cases, lymphadenopathy.

CTCL is more frequent in the elderly black male (55-60 years of age; 2:1 male to female ratio) (89, 92), with an annual incidence of 1:100,000 (93). It is believed that CTCL arises from a combination of environmental (particularly infections) and genetic factors. Exposure to toxic chemicals (88) and infection by *Staphylococcus aureus*, Epstein-Barr virus or cytomegalovirus may have a role in the triggering of MF/SS (89). HLA-B8, HLA-Aw31 and HLA-Aw32 may be also involved. First degree relatives of CTCL patients have higher risk of suffering from the disease as well, suggesting a genetic predisposition (88).

MF/SS patients have also an increased risk of developing secondary malignant diseases (89), probably as a consequence of the invasion of virtually all organs by the malignant T clone in advanced stages (88). The most common cause of death is infection (50%), due to the permanent disruption of the skin barrier and impaired immunological responses (88).

3.2.1.1 Pathogenesis

A chronic antigenic exposure and stimulation of APCs (particularly dendritic cells) leads to an inflammatory response, with release of chemotactic products for both innate and adaptive immune cells, including T lymphocytes. APCs are also able to travel to lymph nodes, leading to the expansion of antigenic-specific effector and memory T-cells that enter the blood stream and the affected skin areas via adhesion molecules, such as E-selectin, further increasing skin homing of T-cells (91). A disturbance in this process may lead to a clonal proliferation of a malignant Th lymphocyte. As the disease progresses, there is a switch in the type of response. In the early stages, there is a dominance of the Th1 cytokine profile, with the production of IL-2 and IL-12. In advanced stages, a switch to a Th2 cytokine

profile allows the disease to escape the antitumor response (91) and leads to increased immunosuppression and susceptibility to infection, with increased production of IL-4, IL-5, IL-10, IL-13, eosinophilia and high levels of immunoglobulin (Ig)E (89). Defects in the expression of the genes *Fas* and *p53* have also been reported, leading to the inhibition of cellular apoptosis (91). The antigenic presentation by APCs, loaded with apoptotic material from CTCL cells, leads to the proliferation of T regulatory cells, with the expression of *FoxP3* and the immunosuppressive cytokine IL-10, further contributing to an immune-incompetent state (94).

Although usually associated with the expansion of CD3⁺CD4⁺ T lymphocytes and mature memory T-cells (CD45RO⁺), MF may present increased levels of CD8⁺ T cells, particularly in early skin lesions. The malignant T lymphocytes have an aberrant downregulation of CD2, CD5 and CD7 markers, and the latter may be associated with disease progression (89).

3.2.1.2 Staging and Treatment

For staging of MF/SS, a tumour-node-metastasis-blood (TNMB) staging system is used (**Table 1**). This staging is dependent on the assessment of skin and lymph node involvement, haematopoietic and metabolic studies, identification and quantification of Sezary cells in blood by morphologic and/or cytometric analysis, and by imagiology in case of lymph node involvement (91). The clinical staging takes into account the TNMB classification system and is also defined as in **Table 1**.

In the cases of early stage MF, in which only the skin is affected and has a favourable prognosis, skin directed therapies such as topical corticosteroids, topical retinoids and phototherapy, are the first line treatment (95). In advanced stages of MF/SS, the treatment is often refractory and the prognosis is unfavourable. Combined topical and systemic therapy, using retinoids, IFN, extracorporeal photopheresis and chemotherapy, are used to reduce tumour burden, delay disease progression and improve QoL of the patient (95).

Table 1. Tumour-node-metastasis-blood staging and clinical staging in cutaneous T cell lymphoma

TNMB staging							
T (skin)		N (lymph node)		M (metastasis)		B (blood)	
T1: Limited patch/plaque (<10% body surface area)		N0: Clinically uninvolved		M0: No visceral involvement		B0: No circulating atypical (Sezary) cells	
T2: Generalized patch/plaque (≥ 10% body surface area)		N1: Clinically abnormal, histologically uninvolved		M1: Visceral involvement		B1: Circulating atypical (Sezary) cells	
T3: Tumours		N2: Clinically uninvolved, histologically involved					
T4: Generalized erythroderma		N3: Clinically abnormal, histologically involved					
Clinical staging							
IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB
T1	T2	T1-2	T3	T4	T4	T1-4	T1-4
N0	N0	N1	N0-1	N0	N1	N2-3	N0-3
M0	M0	M0	M0	M0	M0	M0	M1

The tumour-node-metastasis-blood (TNMB) staging system takes into consideration the involvement and type of lesion in the skin (T), the involvement of the lymph nodes (N), the absence or presence of metastasis (M) and Sezary cells (B).

The clinical staging takes into account the TNMB classification.

Adapted from Kim *et al.* (2005) (91).

3.2.1.3 Pruritus in CTCL

In CTCL patients, pruritus is frequent, chronic, severe and, usually, the earliest, most bothersome symptom (96, 97). As in other haematological malignancies, it is often exacerbated by water and heat. It affects adversely patients' QoL, interfering with sleep, causing low self-esteem and, ultimately, significant physical and mental morbidity (90). Itch is particularly severe in the advanced CTCL variants, folliculotropic and erythrodermic MF, and SS.

The etiology of itch in CTCL is unknown. Mast cells have a role in the skin manifestations of MF, especially in the advanced stage (98). As some mast cell mediators are recognised pruritogens, molecules like histamine might be involved in the pathogenesis of pruritus in CTCL. However, pruritic CTCL patients are often unresponsive to antihistamines (99). It has been recently proposed that itch is a direct result of the Th2 response environment and intense local skin inflammation. Thus, a significant role for cytokines has emerged and IL-31 seems to be a promising mediator of itch in CTCL. IL-31 is produced by Th2 cells, particularly by the malignant T-cell population in MF/SS (46). Its levels correlate with disease severity (43) and resolution of pruritus was associated with the decrease in IL-31 levels (45, 46). A study by Malek and colleagues did not find a relation between pruritus and IL-31 levels in early stage disease (47), probably due to the predominant Th1 environment in this stage of the disease.

Nevertheless, other mechanisms might be involved. It was reported that an antagonist of a SP receptor improved pruritus and QoL in patients with erythrodermic CTCL (30); gabapentin, an anticonvulsant, and mirtazapine, an antidepressant, were successful in managing pruritus in advanced MF/SS (100).

The choice of the treatment for pruritus depends of the stage and severity of CTCL, and of the patient's clinical history. Management of pruritus is often achieved by treating the underlying disease. Thus, topical emollients and phototherapy are of high value in managing itch in early stages of CTCL. The use of systemic therapy in advanced stages is also effective in relieving pruritus and increasing QoL in some of these patients (96).

3.2.2 Mastocytosis

Mast cells (MCs) are effector cells produced by proliferation and differentiation of haematopoietic stem cells that are involved in allergic and other systemic disorders, skin inflammation and cancer. MCs are activated by allergens, complement molecules, cytokines and neuropeptides, immunoglobulins, poison and physical stimuli during host defence and acute inflammatory processes (101, 102). Activated MCs release pre-formed molecules, such as histamine, serotonin, TNF and proteases, and also synthesize *de novo* leukotrienes, prostaglandins, cytokines (IL-1, IL-6, IL-8 and others) and VEGF (103), modulating immune responses and leading to multifunctional tissue responses.

Mastocytosis is a heterogeneous group of clinical disorders characterized by accumulation and activation of aberrant tissue MCs in the skin, bone marrow and other organs. Mastocytosis is initially asymptomatic and may present a chronic indolent course or evolve into a highly aggressive disease with multisystem involvement. Mastocytosis can be confined to the skin, in cutaneous mastocytosis (CM), or may affect other organs, in the

case of systemic mastocytosis (SM). The clinical manifestations of mastocytosis come from MC-derived mediators and from the destructive infiltration of organs by MCs, although the formers seem to have a more preponderant role in the course of the disease (101).

Mastocytosis is a rare disease, despite its increase in the last decades, probably due to better awareness and diagnosis. Epidemiology data is scarce; a recent Danish population-based study found that the prevalence of mastocytosis was 0.89/100 000/year (104).

Mastocytosis may present different clinical features and severity. CM affects only the skin and is more common in children, with the onset around 2 years of age. CM has a good prognosis and the signs and symptoms normally resolve at adolescence. In adults, cutaneous disease is commonly associated with SM (101). Macropapular CM/Urticaria pigmentosa (UP) is the most common type of CM, presenting with red brown macules and papules that induce erythema, wheal and pruritus when scratched. The lesions are symmetrical, with 1 cm of diameter. The life expectancy of UP patients is equal to the general population.

In SM, defined as a MC disease involving at least one extra cutaneous organ, the most of the patients present a D816V mutation and aberrant expression of CD25 molecule (the α -chain of the IL-2 receptor) (101). The most common SM, indolent SM, has a favourable prognosis without decreased life expectancy or organ damage. This variant has a low MC burden in the bone marrow, with a low rate of MC proliferation. The symptoms are, therefore, largely associated with the release of inflammatory cytokines by MCs, such as TNF- α , IL-1 β and IL-6 (101, 105). The symptoms are chronic or episodic and include facial flushing, pruritus, palpitations, nausea, anxiety and depression, among others (101). A very common complication is mastocytosis-associated osteopenia and osteoporosis, which is related to the intensity of MC mediator release (106). The indolent SM patients have also an increased risk of suffering from anaphylaxis episodes, compared with the general population (107). SM with associated clonal haematological non-mast cell lineage disease is an aggressive variant of SM, in which besides the increased proliferation of MCs, the patients also develop a myelodysplastic/myeloproliferative syndrome or acute myeloid leukaemia. The malignant clones, usually, have the D816V mutation along with other mutations. The prognostic is unfavourable, with a significant reduction in patients' survival. The aggressive SM is the most severe form of SM, in which end-organ dysfunction is a direct consequence of MC infiltration of the bone marrow, gastrointestinal tract, liver, spleen and lymph nodes. As occurs in SM with associated clonal haematological non-mast cell lineage disease, aggressive SM has a poor prognosis and a short survival rate.

SM is more common in adults and CM more common in the paediatric population, in which regression is common and usually occurs in puberty (108). Most cases of mastocytosis appear spontaneously, but there are reports of familial cases (109).

3.2.2.1 Pathogenesis

The differentiation and maturation of MCs from haematopoietic stem cells is dependent on the activation of the transmembrane receptor tyrosine kinase (CD117; KIT) by stem cell factor (SCF). The KIT receptor is widely expressed on haematopoietic stem cells of both myeloid and lymphoid lineages and is downregulated in all mature lineages, except in MCs (105). Mastocytosis arises from mutations in the *KIT* gene. The gain-of-function point mutation of the *KIT* gene in which a valine is substituted by an aspartic acid in the catalytic domain of the protein (termed D816V mutation) is the most common and important mutation, although mutations in other codons of the *KIT* gene, and mutations in other genes may also occur. The D816V mutation allows for autophosphorylation of KIT in the absence of SCF, leading to an increase in growth, differentiation, survival and activation of MCs (101, 102). This mutation also allows an increase of MCs proliferation via IgE receptor through common signal pathways, further enhancing the MC burden.

3.2.2.2 Diagnosis and Treatment

Mastocytosis is difficult to diagnose since its symptoms may be nonspecific and mild. Differential diagnosis implies the distinction of mastocytosis from other MC neoplasms and diseases in which there is MC activation. Despite being a rare disease, the presence of characteristic skin lesions and signs and symptoms that relate to MC mediator release, should raise the suspicion of mastocytosis. In children, a histological or genetic evidence, in addition to skin lesions defines CM. In adults with typical skin lesions, a bone marrow examination is necessary to rule out SM. The diagnosis of SM is based on skin lesions, assessment of serum tryptase levels (normal values <20 ng/mL; increased with MC proliferation and activation), histological studies and detection of the genetic mutation in MCs of an extra-cutaneous tissue (110). After diagnosis, imaging techniques aid in the classification of disease variant and severity.

There is no cure for mastocytosis. Disease treatment aims to improve patients' QoL, which is severely impaired, by blocking production and release of inflammatory mediators by MCs. A first therapy approach is the treatment with histamine receptor antagonists, which control skin symptoms, tachycardia and abdominal pain, in patients with SM. A continued therapy with calcium and vitamin D is prescribed to patients with osteoporosis. Leukotriene antagonists are prescribed when histamine receptor antagonists are unsuccessful, and to

manage gastrointestinal and cutaneous complications in paediatric mastocytosis. Other drugs, such as systemic glucocorticoids, the monoclonal antibody omalizumab, phototherapy and cytoreductive drugs, proved to be effective in reducing skin and other symptoms in some patients (111). Additionally, patients are advised to avoid potential triggers of systemic mediator release, as occurs in the case of ingestion of spicy foods, insect bites, emotional stress, exercise practice, among others (101).

3.2.2.3 Itch in mastocytosis

Pruritus is the most common symptom reported by mastocytosis patients and appears to result from MC mediator release.

Histamine seems to be an important mediator in the pruritus of mastocytosis, as histamine receptor antagonists are the first line of treatment for CM and are effective in the treatment of this symptom in some patients (111). However, other molecules may be also involved, such as IL-31, that is largely produced by MCs. Ishii *et al.* demonstrated that in myeloproliferative disorders, pruritus was significantly correlated with MC burden, decreased apoptosis and high IL-31 levels (44). Moreover, IL-31 serum levels were increased in mastocytosis patients and correlated with disease severity, but this enhancement was not associated with pruritus and other skin symptoms (42).

In the early stages of a chronic allergic reaction, skin MCs rapidly produce IL-8, in order to recruit and activate granulocytes (112). Neutrophil chemotactic activity is also increased in mastocytosis patients with pruritus, as compared to non-pruritic patients (113).

Human MCs are capable of producing and releasing serotonin and possess serotonin receptors in their surface (114). Serotonin levels follow a different pattern in mastocytosis patients, compared to healthy subjects, but no correlation was observed between serotonin and pruritus in these patients, regardless of the disease variant (21).

The bone marrow of mastocytosis patients presents increased angiogenesis (53). Neoplastic cells from SM express both VEGF and VEGF receptors mRNA (115). However, Brockow *et al.* found that VEGF serum levels were not increased in mastocytosis patients, compared to atopic dermatitis patients or healthy subjects (116).

Th2 immune responses may have an important role in mastocytosis. In fact, MCs are classically involved in inflammatory responses against helminthic infections, in which Th2-derived cytokines enhance MC proliferation and activation (117). It was reported that IL-3, IL-4 and IL-10 downregulated protein and mRNA levels of KIT in MCs and other haematopoietic cells (118, 119), which, altogether, points to a strict regulation of MCs by Th2 mediators. However, Spínola *et al.* did not find increased circulating levels of basophils or Th2 lymphocytes in mastocytosis patients, in comparison with healthy subjects (120).

3.3 Other systemic disorders

Itch is a frequent symptom in liver disorders, particularly in those that affect the biliary tree. Cholestatic pruritus may occur in pregnant women, may be drug-induced (121, 122) or caused by viral infections (hepatitis B and C). The vast majority of Human Immunodeficiency Virus (HIV) patients complain of pruritus during some point in the course of the disease either due to the infection itself (123) or to a secondary condition, such as a dermatological disorder (124).

3.4 Neurological/Psychiatric disorders

Neuropathic itch is present in various conditions of the nervous system and occurs without stimulation by a pruritogen. Itch has been reported in multiple sclerosis, spinal tumours, depression, and other dysfunctions of the central and peripheral nervous system (125).

4 Evaluation of itch and treatment

Itch is a highly underestimated condition. Indeed, despite the prevalence of itch in dermatologic disorders and in other pathologies, few studies address the prevalence of itch in European countries (1). Studies performed in Norway (126) and Germany (127) reported that chronic itch appears to be more frequent in adult women, with poor well-being (up to 20% had depression). Surprisingly, near half of the patients complaining of high intensity pruritus that impaired their QoL did not seek for medical advice. More studies are necessary to assert the influence of these and other variables in the occurrence of itch. Probably, one of the reasons to the scarce epidemiology data, is that patients don't seek a physician. This may be due to the organization of the healthcare system or it may be a cultural or educational aspect.

In 2014, fifteen European countries united efforts to improve the assessment and treatment of chronic pruritus in dermatological care, by developing the European Network on Assessment of Severity and Burden of Pruritus (PruNet) (128). Their main goal was to evaluate several Patient-reported Outcome (PRO) tools used in the diagnosis of itch in adults, in order to unify criteria and pursue guidelines. The investigation of the intensity of pruritus and the impact of itch in the patient's QoL were the most important tools to evaluate pruritus. Although it is not validated in most countries, the visual analogue scale (VAS) is the most used tool to assess the intensity of pruritus (128). It consists of a 10 cm line with a scale from 0 to 10 in which the patients cross the scale in the point that correlates with the intensity of their pruritus: the beginning of the scale (0 points) indicates no pruritus, and the end (10 points) of the scale, the most severe pruritus (129). Although VAS is a reliable tool to assess the intensity of pruritus, it has several limitations: it may be influenced by circadian variation and is not suitable for patients with motor or cognitive disorders that prevent them from either using a pen or understand the VAS (129). New tools on evaluating itch are being developed to overcome these difficulties and to assess the impact of itch in the patient's QoL. One example is the ItchyQoL, a pruritus-specific QoL questionnaire, that evaluates patients' symptoms, functional limitations and emotions (130).

Regarding treatment, patients often see their pruritus unrelieved or mildly attenuated by the drugs currently available. These drugs include histamine receptor antagonists, selective serotonin reuptake inhibitors, inhibitors of arachidonic acid pathways, SP inhibitors, and immunosuppressive drugs. In spite of the availability of these itch-specific treatments, patients with the same underlying disease may respond differently to the same treatment.

Aims

Chronic pruritus is a major and distressing symptom common to several dermatological and haematological conditions. Pruritus arises from a complex dialogue between skin cells and neurons from both the peripheral and central nervous system. This dialogue appears to be sustained by mediators such as cytokines, growth factors, neurotransmitters and other molecules. Indeed, mechanisms underlying itch are complex and seem to involve, for the different diseases, different pathways, cells and mediators.

Pruritus is a common symptom in CTCL, mastocytosis and psoriasis, having a significant impairment in patients' health-related QoL. The immune response seems to have a crucial role in these inflammatory conditions, raising the hypothesis that some cytokines and growth factors may be important for the inflammatory response and for pruritus. The response to similar therapeutic strategies to treat these pruritus-associated diseases is often different, strengthening the need to clarify the underlying mechanisms of pruritus in these skin diseases and to identify potential therapeutic targets. This study aims to clarify the pathways and mediators that may trigger pruritus in CTCL, mastocytosis and psoriasis patients, by evaluating different cytokines (IL-8, IL-31 and IL-33), neuromediators (serotonin), endothelial adhesion molecules (E-selectin) and angiogenic factors (VEGF). For each disease under study, the correlation of the several biomarkers with itching score and severity of the disease will be studied. By clarifying the mediators involved and by establishing their relationship with pruritus, we expect to contribute to a better knowledge on the mechanisms triggering pruritus, and to identify potential therapeutic targets.

Materials and Methods

1 Subjects

The scientific and ethic committees of University Hospital of Coimbra (CHUC) and Hospital Santo António from Centro Hospitalar do Porto (CHP) approved this study. All patients and controls provided written informed consent.

The control group included 33 apparently healthy volunteers (blood donors) with normal haematological and biochemical values. Adult psoriasis *vulgaris* patients (n=30), from CHUC and CHP had moderate to severe disease, according to the PASI score. Moderate psoriasis patients (n=16) presented a PASI score below 20, and a PASI score >20 was indicative of severe disease (n=14). None of the psoriasis patients received any treatment for at least one month prior to inclusion. CTCL patients (n=29) from CHP were adults, with either MF (n=17), SS (n=10) or unclassified CTCL (n=2). Adult mastocytosis patients (n=17) were from CHP.

Patients and controls presenting other skin diseases, inflammatory or infectious diseases and liver or kidney diseases were excluded from the study. Controls and patients were not under any dietary restriction or therapy that could interfere with the results.

Pruritus was auto-evaluated in CTCL and mastocytosis patients using a number scale (0-10) similar to VAS. In psoriasis patients, the pruritus felt in the preceding week was auto-evaluated in a scale of 0 to 3. This evaluation is included in the first question of DLQI questionnaire (**Annex 1**) “Over the last week, how itchy, sore, painful or stinging has your skin been?”.

2 Sample collection

Blood from nonfasted subjects was collected in order to obtain whole blood (EDTA as anticoagulant) and serum. None of the collected samples was icteric or haemolysed. Serum aliquots were stored at -80°C until analysis.

3 Analytical studies

A complete blood count was performed using an automatic haematological counter (LH780, Coulter, California, USA).

IL-8, IL-31 (BioLegend, California, USA), IL-33, VEGF, E-selectin, (eBioscience, California, USA), C-reactive protein (CRP; R&D Systems, Minneapolis, USA) and serotonin (Enzo Life Sciences, New York, USA) were quantified in serum, using enzyme linked immunosorbent assays (ELISA), according to the manufacturer’s instructions.

In mastocytosis patients, serum tryptase levels were evaluated by a Fluoro Immuno Enzymatic Assay (ImmunoCap, ThermoFisher Scientific, Massachusetts, USA). Tryptase levels were considered elevated when above 20 ng/mL.

4 Statistical analysis

All statistical analyses were performed in IBM SPSS Statistics 23 for Windows (IBM Corporation, New York, USA). A Kolmogorov-Smirnov ($n > 50$) or Shapiro-Wilk ($n < 50$) test was used to determine the distribution of the variables. Data is presented as median levels (interquartile range, IQR), as all variables follow a non-parametric distribution. To evaluate differences between groups, the Kruskal–Wallis H test was used and, when statistical significance was achieved, single comparisons (two groups) were made by using the Mann–Whitney U test. Spearman's rank correlation coefficient was used to evaluate relationships between sets of data. Adjustment of statistical differences for confounding factors was performed by analysis of covariance (ANCOVA). For that analysis, non-parametric data was transformed to parametric data, using the method described by Templeton (131).

Graphical representations were made using GraphPad Prism 7.01 (GraphPad Software, Inc., California, USA).

A *P* value < 0.05 was considered statistically significant

Results

Our results were analysed in two steps, firstly by comparing each group of patients with the control group and by comparing CTCL, mastocytosis and psoriasis patients. This was followed by a subanalysis, comparing the CTCL patients with MF with those presenting SS; comparisons between moderate and severe psoriasis patients were also performed.

In **Table 2**, demographic and clinical data for controls and patients is presented. Patients and controls were not matched for age; in the case of mastocytosis patients, they were not matched for gender with controls. Thus, as previously referred, an adjustment for age and gender of the studied variables was made. All differences kept their significance after this adjustment.

Table 2. Demographic and clinical data for controls and patients

		Control (n=33)	CTCL (n=29)	Mastocytosis (n=17)	Psoriasis (n=30)
Age (years)		58 (54-61)	66 (55-74) *	43 (36-58) ** ●	50 (31-57) *** ●
Gender	M (n/%)	22/66.7	15/51.7	5/29.4 *	13/43.3
	F (n/%)	11/33.3	14/48.3	12/70.6 *	17/56.7
Pruritus (VAS)		-	1 (0-9)	1 (0-2)	-
Pruritus (1st question DLQI)¹		-	-	-	2 (1-3)
PASI		-	-	-	18.9 (12.0-24.9)

Pruritus was evaluated by a method analogous to the visual analogue scale (VAS) in cutaneous T-cell lymphoma (CTCL) and mastocytosis patients and by the first question of the dermatology life questionnaire index (DLQI) in psoriasis patients¹.

¹ "Over the last week, how itchy, sore, painful or stinging has your skin been?"

M, male; F, female; PASI, psoriasis area and severity index.

*P vs control <0.05; **P vs control <0.01; ***P vs control ≤0.001; ● P vs lymphoma ≤0.001

The total leucocyte, neutrophil, lymphocyte and monocyte counts of controls and patients are presented in **Table 3**. No significant changes were observed.

Table 3. Haematological studies in control and patients

	Control (n=33)	CTCL (n=29)	Mastocytosis (n=17)	Psoriasis (n=30)
WBC (x10⁹/L)	6.3 (5.4-7.9)	6.6 (5.7-8.0)	7.9 (5.6-9.1)	7.3 (6.0-8.1)
Neutrophils (x10⁹/L)	-	4.3 (3.4-4.8)	4.5 (3.0-6.5)	4.7 (3.6-5.3)
Lymphocytes (x10⁹/L)	-	2.1 (1.2-2.8)	2.3 (1.7-2.8)	1.8 (1.5-2.0)
Monocytes (x10⁹/L)	-	0.5 (0.4-0.6)	0.4 (0.3-0.7)	0.6 (0.4-0.6)

CTCL, cutaneous T-cell lymphoma; WBC, white blood cell.

Concerning the biochemical studies (**Table 4**), we found that, compared to controls, the three groups of patients showed significantly higher levels of CRP ($P=0.008$, $P=0.038$ and $P<0.001$, for CTCL, mastocytosis and psoriasis patients, respectively). Serotonin was significantly increased in CTCL and mastocytosis patients ($P=0.049$ and $P=0.005$, respectively). E-selectin was significantly enhanced in CTCL ($P=0.043$), and in psoriasis patients ($P<0.001$). IL-31 levels showed a strong trend towards an increase ($P=0.055$) in CTCL patients. The levels of IL-8 ($P=0.043$) and VEGF ($P=0.005$) were significantly higher in psoriasis patients.

Table 4 Biochemical studies for controls and patients

	Control (n=33)	CTCL (n=29)	Mastocytosis (n=17)	Psoriasis (n=30)
IL-8 (pg/mL)	8.7 (4.8-20.6)	10.2 (4.8-27.9)	8.0 (4.8-36.8)	14.1 (7.9-82.4) *
IL-31 (pg/mL)	9.3 (1.2-20.8)	17.3 (6.1-28.3)	6.8 (2.3-30.4)	10.7 (4.1-29.0)
Serotonin (ng/mL)	164.6 (110.7-197.8)	182.2 (160.6-239.9) *	239.5 (155.0-260.6) **	140.7 (33.1-191.3) ● ◇◇
E-selectin (ng/mL)	38.5 (28.1-43.7)	44.0 (31.2-84.3) *	36.1 (28.5-53.7)	56.8 (44.1-81.4) *** ◇◇
VEGF (pg/mL)	588 (472-725)	653 (429-879)	849 (440-1243)	841 (541-1117) **
CRP (µg/mL)	0.7 (0.3-0.9)	1.4 (0.5-5.5) *	1.2 (0.4-3.9) *	2.0 (0.7-4.7) ***
Tryptase (ng/mL)	-	-	23.7 (10.1-49.3)	-

Tryptase levels, a marker for mast cell activation, were measured only in mastocytosis patients.

CTCL, cutaneous T-cell lymphoma; IL, interleukin; VEGF, vascular endothelial growth factor; CRP, C-reactive protein.

* P vs control <0.05 ; ** P vs control <0.01 , *** P vs control ≤ 0.001 ; ● P vs lymphoma <0.05 ; ◇◇ P vs mastocytosis <0.01

In CTCL patients, E-selectin presented a significant and positive correlation with CRP levels ($r=0.613$, $P<0.001$) and pruritus intensity ($r=0.564$, $P=0.001$); pruritus was also significantly and positively correlated with age ($r=0.511$, $P=0.005$). Moreover, CTCL patients with pruritus ($n=18$) were significantly older than non-pruritic ones ($n=11$) ([median (IQR)] 70 (55-74) and 59 (51-69), respectively; $P=0.040$). We also observed that the levels of IL-31 were significantly higher in CTCL patients with pruritus than in CTCL patients without that symptom ([median (IQR)] 25.1 (8.3-38.6) vs 10.3 (1.8-21.9), respectively; $P=0.028$).

Considering CTCL variants, we found a three-fold increase in IL-31, a two-fold increase in E-selectin and a five-fold increase in the levels of CRP in SS patients, as compared to MF patients ($P=0.003$, $P=0.021$ and $P=0.029$, respectively) and to control group ($P=0.001$, $P=0.002$, $P<0.001$, respectively) (**Figure 1A-C**). Pruritus was also significantly more severe in SS compared to MF patients ($P=0.001$) (**Figure 1D**). SS patients presented significant and positive correlations between CRP and E-selectin ($r=0.891$, $P=0.001$) and the same type of correlation was observed between E-selectin and pruritus ($r=0.820$, $P=0.004$) and between IL-8 and VEGF ($r=0.900$, $P<0.001$).

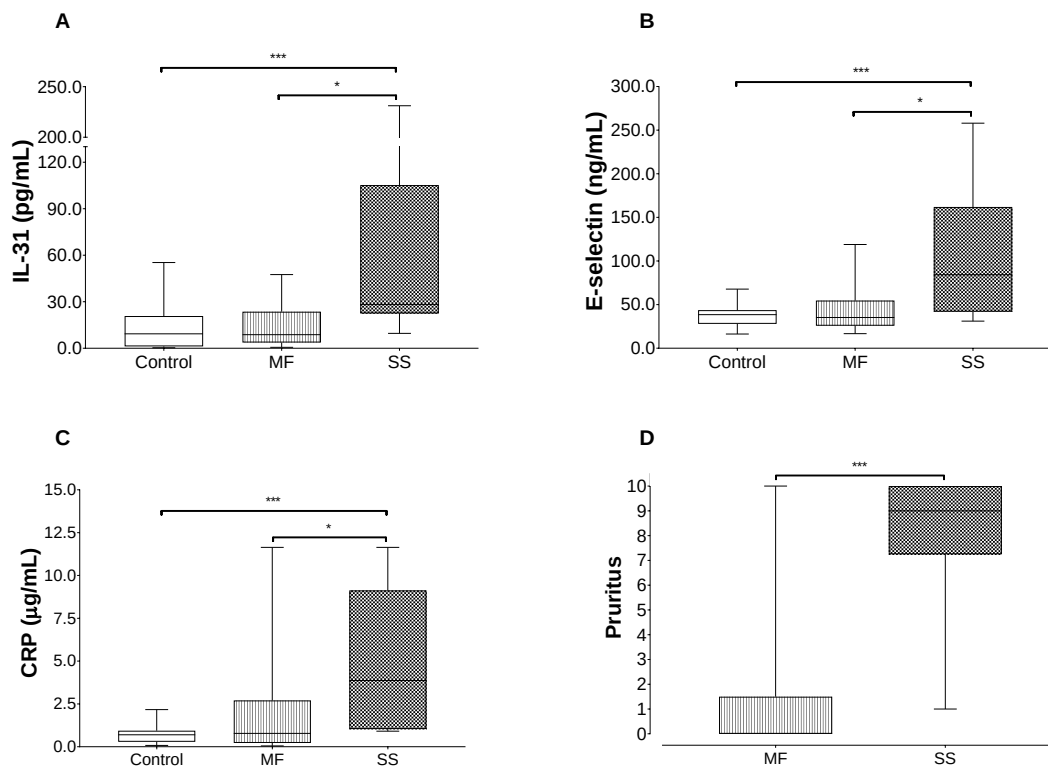


Figure 1. Differences in studied biomarkers between controls, mycosis fungoides and Sezary syndrome patients.

Interleukin (IL)-31 (**A**), E-selectin (**B**) and C-reactive protein (CRP) (**C**) levels were assessed by ELISA in Mycosis Fungoides (MF; $n=17$) and Sezary Syndrome (SS; $n=10$) patients, and controls ($n=33$). The intensity of pruritus (**D**) was evaluated by VAS in MF and SS patients.

* $P < 0.05$; *** $P \leq 0.001$.

There were no statistical significant differences in studied parameters between MF patients and controls. However, there was a trend towards an increase in the serotonin levels ($P=0.057$). Moreover, in MF patients, IL-31 correlated significantly and positively with CRP ($r=0.487$, $P=0.047$).

In mastocytosis patients, we found a positive and significant correlation between the severity of pruritus and the levels of tryptase ($r=0.670$, $P=0.009$). Indeed, patients with pruritus ($n=10$) had significant higher levels of tryptase than those that did not complain of this symptom ($n=7$) ([median (IQR)] 42.6 (14.0-177.6) and 9.0 (3.4-34.0), respectively; $P=0.020$).

In psoriasis, IL-8 correlated significantly and positively with E-selectin, VEGF and CRP ($r=0.413$, $P=0.023$; $r=0.393$, $P=0.032$ and $r=0.443$, $P=0.016$, respectively) (**Figure 2A-C**).

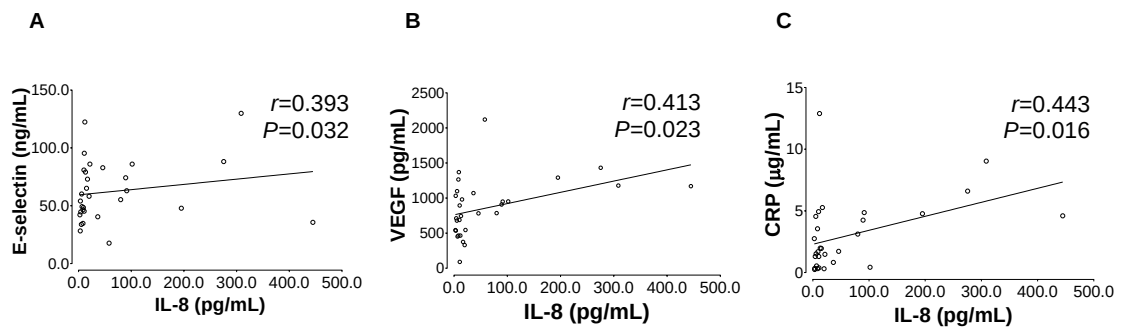


Figure 2. Correlation between studied biomarkers in psoriasis patients.

Serum levels of interleukin (IL)-8, E-selectin (**A**), vascular endothelial growth factor (VEGF) (**B**) and C-reactive protein (CRP) (**C**) were measured by ELISA in psoriasis patients ($n=30$).

Moreover, serotonin levels showed a significant and positive correlation with IL-31 and VEGF ($r=0.373$, $P=0.042$ and $r=0.500$, $P=0.005$, respectively); positive and significant correlations were also found between CRP and the total leucocyte ($r=0.597$, $P=0.001$), neutrophil ($r=0.680$, $P<0.001$) and monocyte ($r=0.486$, $P=0.016$) counts, as well as with PASI score ($r=0.556$, $P=0.002$), in psoriasis patients.

When psoriasis patients were divided according to severity of disease, considering PASI score, we found that severe psoriasis patients presented significantly higher levels of IL-8 ($P=0.019$), E-selectin ($P<0.001$), and VEGF ($P=0.002$), as compared to controls (**Figure 3A-C**); CRP levels were also higher in severe patients as compared to controls ($P<0.001$) and to patients with moderate psoriasis ($P=0.003$) (**Figure 3D**). Moderate psoriasis patients presented also significantly higher levels of E-selectin, when compared to control subjects ($P=0.004$) (**Figure 3B**).

We evaluated also IL-33, for all the groups studied, however, all the results were below the detection line.

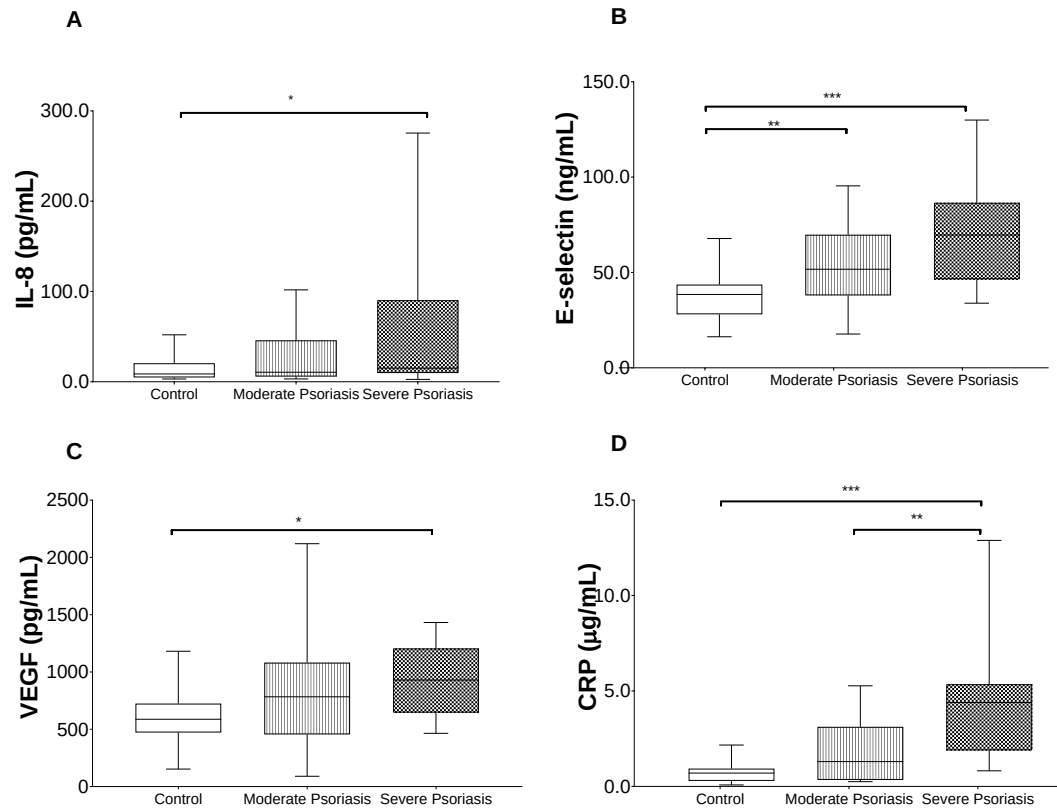


Figure 3. Differences in studied biomarkers between controls and patients with moderate and severe psoriasis.

Interleukin (IL)-8 (A), E-selectin (B), vascular endothelial growth factor (VEGF) (C) and C-reactive protein (CRP) (D) levels in moderate (n=16) and severe psoriasis patients (n=14), and controls (n=33).

*P < 0.05; **P < 0.01; ***P ≤ 0.001.

Discussion

The mechanisms underlying pruritus are complex and seem to involve, for different diseases, different pathways, cells and mediators. **Figure 4** aims to illustrate the intricate pathways in the pathogenesis of CTCL, mastocytosis and psoriasis and its relations to pruritus. In the three diseases, skin damage activates antigen presenting cells, which release inflammatory cytokines (in particular IL-8), recruiting more circulating neutrophils and T-lymphocytes (1). APCs are also able to travel to the nearest lymph node (2), allowing expansion of antigenic-specific effector T-cells that travel through the blood stream (3) and enter the affected area via adhesion molecules, such as E-selectin; neutrophils are also capable to recruit T-lymphocytes, further increasing their homing to the skin (4). T lymphocytes may undergo phenotype switch, according to cytokine environment (5): Th1 and Th17 cells, involved in the pathogenesis of psoriasis, activate macrophages and dendritic cells through the production of IFN, TNF and IL-17 (6), which, together with neutrophils, are able to activate keratinocytes (7). Activated keratinocytes produce VEGF and IL-8, enhancing vascularization of the tissue and recruitment of more immune cells (8).

In CTCL, particularly, as disease evolves from MF to the more aggressive SS, a Th1 cytokine profile switches to a Th2 cytokine profile (9).

Th2 lymphocytes are able to modulate the metabolism of MCs, through the release of Th2 cytokines (IL-3 and IL-4) (10). In mastocytosis, the malignant MCs undergo clonal expansion, with release of mediators, including IL-8, IL-31 and VEGF. IL-8 is chemotactic for neutrophils and VEGF induces angiogenesis and vascularization (11).

The pool of cytokines involved in the pathogenesis of these diseases is present not only in lesioned areas, but has also a systemic spread.

Pruritus perception begins in the neuronal pathways of pruriceptive information of the epidermis (top left). Briefly, unmyelinated C-fibres and A δ -fibres sense pruritic stimuli. Sensory input is then integrated into electrical information, conducted to dorsal root ganglion neurons. From the spinal cord, second-order neurons synapse with STT neurons that pass through the thalamus and in to the somatic sensory cortex, where information is processed.

Pruritus is a difficult symptom to treat; indeed, different diseases, as well as different clinical stages of the same disease, may respond differently to the same itch treatment. Our results are in line with this, showing that pruritus, in CTCL, mastocytosis and psoriasis, involves different mediators, and, thus, it is likely that it involves different pathways and, therefore, different therapeutical targets should be considered.

CTCL, mastocytosis and psoriasis are all associated with inflammation, as shown by the increased levels of CRP found in the patients, as compared to controls.

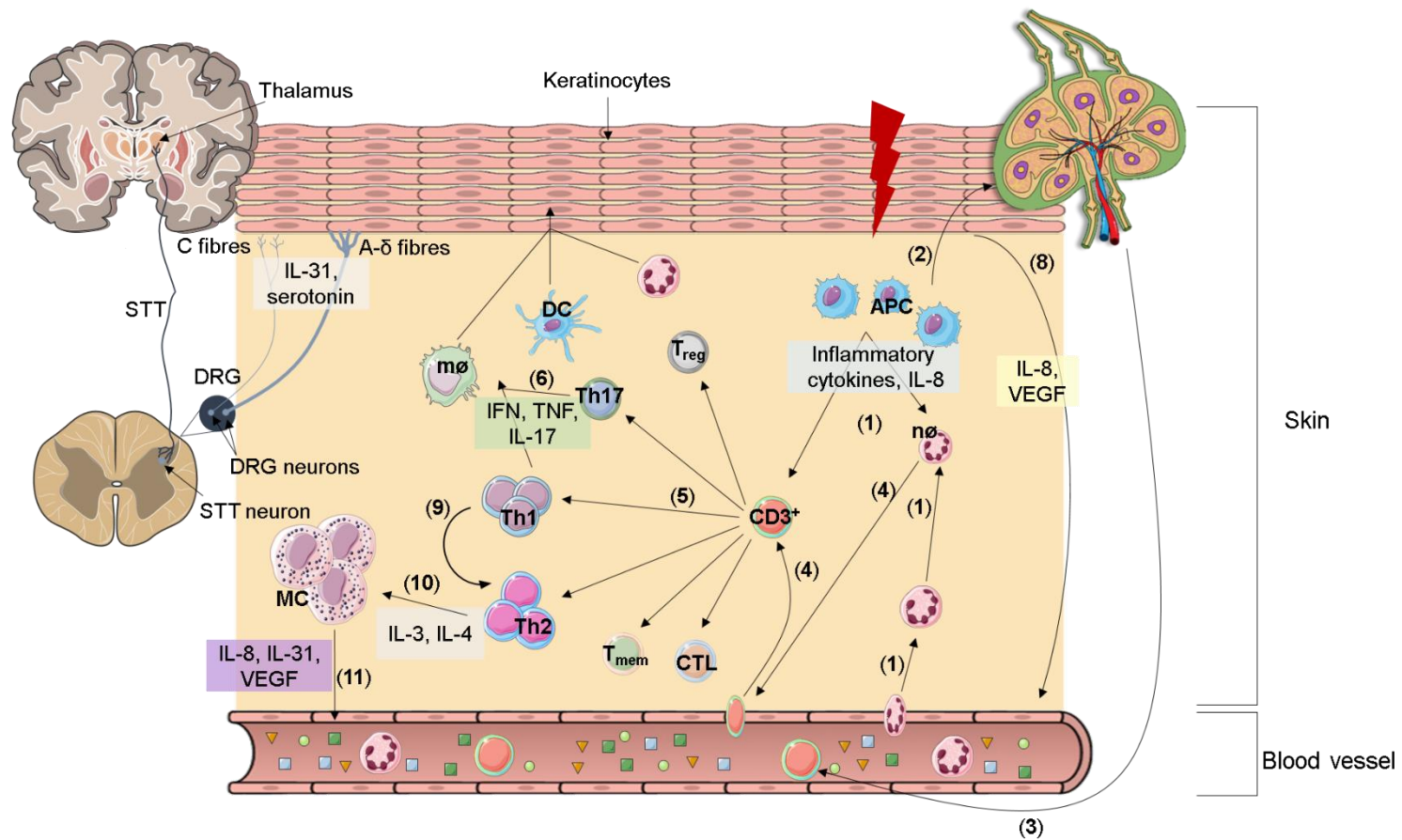


Figure 4. Pathogenesis and itch mechanisms in cutaneous T cell lymphoma, mastocytosis and psoriasis.

See text for description

APC, antigen presenting cell; CTL, cytotoxic T lymphocyte; DC, dendritic cell; DRG, dorsal root ganglion; IFN, interferon; IL, interleukin; MC, mast cell; mØ, macrophage; nØ, neutrophil; CD3⁺, T lymphocyte; T_{mem}, memory T cell; Th, T helper cell; T_{reg}, regulatory T cell; TNF, tumour necrosis factor; STT, spinothalamic tract; VEGF, vascular endothelial growth factor; the squares, circles and triangles inside the blood vessel represent the systemic spread of the molecules involved in the pathogenesis of the three diseases.

In CTCL, itch is a frequent, chronic and severe symptom, interfering with patients' QoL since early stages. In the present study, we found that CTCL patients presented significantly higher levels of E-selectin and serotonin, as compared with controls. Also, and as previously mentioned, several studies reported that IL-31 levels correlate with pruritus in CTCL patients (45, 46). We also found a significant correlation between pruritus and IL-31 in CTCL patients; moreover, CTCL patients with pruritus showed significantly higher levels of IL-31, compared with non-pruritic CTCL patients, although IL-31 blood levels were not significantly higher than in controls.

IL-31 is produced by the malignant T cell clone (46) and, in mice skin, homing of T lymphocytes from SS patients was mediated by E-selectin (51). Our data are in accordance with these findings, suggesting that IL-31 and E-selectin are important players in the mechanisms underlying pruritus in CTCL, especially in SS. The high levels of IL-31 are likely to result from an overwhelming production by T cell malignant clone, and the elevated levels of E-selectin may contribute to the development of erythroderma, observed in SS patients.

We also found, in CTCL, a significant correlation between age and itch, showing that elderly patients are more prone to suffer from pruritus, and, consequently, this should be considered when treating this symptom in old-age patients.

CTCL includes a complex array of diseases, namely the variants MF and SS, with different clinical manifestations and therapeutic responses.

MF, usually, presents as a mild and localized form of CTCL. Actually, our data showed that the levels of the studied biomarkers in MF patients were very similar to those of the control group; they presented, however, a tendency for higher levels of serotonin. Studies using serotonin re-uptake inhibitors showed significant improvement of pruritus in various diseases, including haematological malignancies (22, 132), indicating that serotonin agonists may be a potential solution for the treatment of this symptom. On the same note, the serotonin agonist mirtazapine was effective in treating itch in MF/SS patients (100). Thus, the increase in serotonin levels seems to be beneficial to the management of this symptom, but more studies are necessary to clarify its role in CTCL-associated itch.

SS is a systemic and more aggressive form of CTCL than MF. We found that pruritus intensity was higher in SS patients, as compared to MF. According to our findings, IL-31, E-selectin and CRP levels were significantly enhanced in SS patients, comparatively to MF patients, showing that the inflammatory process is more pronounced in SS. E-selectin correlated with the inflammatory marker CRP, and with pruritus intensity. Our data suggest an important role for E-selectin, inflammation and for IL-31 in the development of itch in

these patients. In fact, a study using a monoclonal antibody against IL-31 diminished scratching behaviour in a mouse model of atopic dermatitis (133), and Cedeno-Laurent *et al.* demonstrated that disruption of IL-31 production by malignant T lymphocytes successfully abolished pruritus in SS patients (45).

Regarding E-selectin, a study measuring this adhesion molecule in the serum of CTCL patients with skin lesions, including MF and SS, did not find increased levels of E-selectin, compared to controls (134); however, the same study reported that E-selectin correlated positively and significantly with lactate dehydrogenase, a marker of poorer prognosis in CTCL, and that is increased in the more aggressive forms of the disease (135). This controversial data about E-selectin may result from the inclusion of MF and SS patients in the same study population. Actually, E-selectin values seem to reflect the severity of inflammation and pruritus, which is lower in MF patients.

In summary, E-selectin and IL-31 seem to be important biomarkers underlying pruritus in SS patients, but less important in MF patients, in whom serotonin may play a more significant role in triggering itch.

Mastocytosis is characterized by high levels of MCs, both in circulation and disseminated through body organs. In this study, in agreement with others (136), mastocytosis patients presented higher levels of serotonin, although, as Kushnir-Sukhov *et al.* demonstrated (21), no significant correlation was found between serotonin values and pruritus.

The pruritic effect of tryptase was already demonstrated in mice (137), and in itch of atopic dermatitis patients (138). Accordingly, we found in mastocytosis patients a positive correlation between pruritus and tryptase levels. Tryptase is a marker of MC burden and activation, and this correlation suggests that pruritus is linked to MC-mediator release. Strengthening this linkage, we found significantly higher levels of tryptase in mastocytosis patients with pruritus, as compared with patients who did not present that symptom. However, when patients were divided according to levels of tryptase (< and > 20 ng/mL), we did not find a significant difference in pruritus intensity between the two groups. This may result from the low number of patients in each group. More studies are needed to clarify the mediators and pathways implied in pruritus in mastocytosis.

Psoriasis is a chronic and inflammatory skin disease, in which itch is a common skin manifestation. In psoriasis patients, we measured skin manifestations using the first question of the DLQI questionnaire (**Annex 1**), asking “Over the last week, how itchy, sore, painful or stinging has your skin been?”. As this is a less accurate evaluation of pruritus than VAS, it may explain why we did not find a correlation between itch and the significantly elevated levels of E-selectin, as was previously reported in psoriasis (32), and with the

levels of VEGF, in prurigo simplex, an inflammatory skin condition that presents with intense itch (55). Moreover, no significant correlations were observed between the pruritus score and the other biomarkers studied, suggesting that they may not be related to itch mechanism in psoriasis. Actually, further studies, evaluating pruritus with a more accurate tool, are necessary to clarify the value of those biomarkers in the genesis of itch in psoriasis. The levels of IL-8, E-selectin, VEGF and CRP were found to be elevated in this condition, which translate the clinical manifestations and histology of psoriasis. Indeed, the elevated levels of IL-8, a chemoattractant for neutrophils, and E-selectin, an adhesion molecule, explain the neutrophilic infiltrate common in psoriatic lesions histology. The high values of VEGF account for the intense local angiogenesis/vascularization, which confers a reddish colour to the cutaneous lesions. Serum E-selectin was also previously demonstrated to be increased in psoriasis patients and to be correlated with PASI score (139), and, although we did not find that correlation, we found statistically significant differences in E-selectin levels between patients with moderate and severe disease, and the control group. Nakamura *et al.* (32) also reported a two-fold increase in this endothelial adhesion molecule in psoriatic skin with pruritus, compared to non-pruritic lesioned skin, which further strengthens the need for clarification of the relationship between pruritus and this and other biomarkers. Our group has already reported, in a previous work, that IL-8 and VEGF levels are elevated in psoriasis patients (35), as well as CRP values. Indeed, we proposed this acute phase protein as a possible biomarker of psoriasis severity (140), in agreement with the results of the present study.

In opposition to Narbutt *et al.* (84), but in accordance with Czarnecka-Operacz *et al.* (141) studies, we did not find a role for IL-31 in itch in psoriasis. The existence of higher levels of IL-31 and serotonin in CTCL and mastocytosis patients, as compared to controls, but not in psoriasis patients, may be related to the type of immune response. Psoriasis presents a Th1/17 immune response (66), whereas in the early stages of CTCL there is a dominance of the Th1 profile, and in advanced stages occurs a switch to a Th2 profile; in mastocytosis, the Th2 pathway seems to have an important role (46, 116).

In summary, our data suggests that serotonin and tryptase are important biomarkers in mastocytosis, but further studies are needed to identify itch-mechanisms in this condition. The high levels of IL-8, E-selectin, VEGF and CRP in psoriasis, especially of E-selectin and CRP in the severer forms of the disease, suggest that they are potential biomarkers of psoriasis severity, and further studies are needed to clarify their involvement in itch mechanisms. In CTCL patients with the more aggressive form, SS, E-selectin and IL-31

appear to be important in itchy pathways, while in MF patients, the increase in serotonin seems to be more important in the mechanisms underlying pruritus.

Our data strengthens the hypothesis that different mechanisms underlie the genesis of itch in these diseases, explaining the different therapeutic responses to common treatments, and proposes some potential therapeutic targets for the treatment of itch in CTCL, mastocytosis and psoriasis patients.

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Annex 1

DERMATOLOGY LIFE QUALITY INDEX

DLQI

Hospital No:

Date:

Name:

Score:

Address:

Diagnosis:

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.

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