MYTHS AND FACTS IN MASTOCYTOSIS

Studying dilemmas in the care for patients with mastocytosis

Maud Hermans

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MYTHES EN FEITEN VAN MASTOCYTOSE

Studies over klinische dilemma's in de zorg voor
patiënten met mastocytose

Maud Anna Wilhelmina Hermans

The studies described in the thesis were performed at the Department of Internal Medicine and Department of Immunology, Erasmus MC, University Medical Center Rotterdam, the Netherlands

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Chapter 1

General introduction and aims of this thesis

1.1 General introduction

Mast cells

Mast cells are pluripotent, long-lived leukocytes that reside in connective tissue and mucosa [1]. It is assumed that mast cells originate from CD34+ myeloid progenitors cells, although a recent publication hypothesized that the hematopoietic progenitor cells might be more flexible than was previously thought and that mast cells can also be phylogenetically related to cells of the lymphoid lineage [2]. Mast cell progenitors leave the bone marrow and mature in other tissues such as skin and mucosa, were they will remain for the rest of their lifespan. Roughly, two subtypes of mast cells are recognized: MC_{TC} who produce the enzymes tryptase and chymase, and MC_{T} who produce tryptase but not chymase [3]. MC_{TC} are typically found in the mucosa of the gastrointestinal tract, whereas MC_{T} are more prominent in connective tissue. Mast cells retain a certain level of plasticity after they have matured, and their phenotype can be influenced by their environment to an important extent [3]. They are one of a few cell types which express the KIT-receptor: A single chain transmembrane receptor with intrinsic tyrosine kinase activity that is essential to cell proliferation and survival and is activated by its ligand stem cell factor (SCF) [4].

Mast cells are part of the innate immune system and play a role in the first line of defense against pathogens from outside, matching their location in barrier sites of the body [5]. It is therefore important that they can react to many different stimuli, including pathogen related molecules, venoms and drugs, as well as signaling substances produced by other human cells such as interleukins and hormones (figure 1) [3]. Physical triggers such as abrupt temperature shifts and mechanical stress can also induce mast cell activation [6]. To enable the mast cell to respond to so many different triggers, it can express many different receptors, each with their own intracellular pathway and subsequent action of the cell [7]. The most well-known route of mast cell activation is through the cross-linking of IgE that is bound to the high-affinity IgE receptor FcεR1 [8]. This route of activation is pivotal in allergy, and it has been researched extensively since the discovery of IgE in the ‘60s [9]. Anoth-

er activating receptor that has gained more interest in the last decade is the Mas-related G-protein coupled receptor 2 (MRGPRX2), which is rather specific to mast cells although it can be found on eosinophils and basophils as well [10]. MRGPRX2 responds to a remarkable amount of possible ligands including small molecule drugs, venoms, certain hormones and neuropeptides such as substance P [11]. The MRGPRX2 receptor has been linked to non-IgE mediated drug and venom hypersensitivity, and is also upregulated in patients with chronic spontaneous urticaria [12]. The fact that activation of MRGPRX2 can lead to symptoms that are very similar to allergic reactions, but without involvement of IgE, has made it a topic of interest for mast cell researchers in recent years [13]. The role of the MRGPRX2 receptor in mastocytosis is still unknown.

Upon activation, mast cells immediately expel preformed mediators by degranulation (figure 1). The type of mediators that are released is dependent on the route of activation [8]. Well-known mast cell mediators that are involved in anaphylaxis are histamine, leukotrienes and prostaglandins [7] but many more mediators can be released upon degranulation. Furthermore, different types of proteases are released that are involved in wound healing but also in the defense to toxic venoms [14]. Of these proteases, tryptase is the most well-known in clinical practice because it can be measured relatively easily in blood and is often used as a biomarker for mast cell activation. In mastocytosis, serum tryptase levels are correlated to the mast cell load in the body, arguing for a basic level of mast cell activity at all times [15]. Shortly after degranulation, the production of pro-inflammatory cytokines is started, which is a slower process that takes up to 24 hours [16].

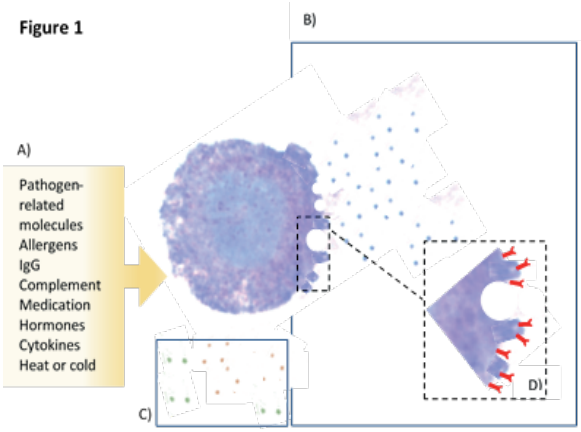


Figure 1. Schematic overview of mast cell degranulation. A) activators of MCs are listed, that can cause mast cell activation. The release of these mediators will result in modulation of biological processes. Cartoon is constructed from microscopic toluidine blue staining of human mast cells. **B)** MC degranulation results in mediator release, among them β-hexosaminidase. Also, as depicted in the doted square, CD63 is expressed on the extracellular membrane. Both β-hexosaminidase release and CD63 expression are read-out systems for MC activation. **C)** Another reaction upon activation of MCs, is the release of mediators such as cytokines, chemokines and lipid mediators.
Credits: master thesis A.C. van Stigt 2019.

Downstream of every receptor is an intracellular cascade of activating and inhibiting molecules that together orchestrate a tailored response. Although papers and books typically depict one pathway downstream of one receptor, in real life, different pathways communicate with each other, and different stimuli will approach a mast cell simultaneously. This makes it difficult to reliably study mast cell biology, or any other immune cell for that matter, *in vitro*. One of the signaling cascades that has multiple functions in mast cells is the Janus-Kinase-2 (JAK2) and Signal Transducer and Activator of Transcription 5 (STAT5) route. The JAK-STAT routes are relatively uncomplicated: Upon activation of a receptor, JAK2 that is docked to the intracellular domain of the receptor is phosphorylated by kinases of the receptor itself. Phosphorylated JAK2 forms a dimer and subsequently phosphorylates other molecules present in the cytosol, including STAT5. Phosphorylated STAT5 then translocates to the cell nucleus, as monomer or dimer, and functions as a transcription factor [17]. It is well known that STAT5 is often constitutively activated in myeloid malignancies, and this has also been demonstrated in mastocytosis, where it is one of the main effectors downstream of the KIT receptor [18]. To illustrate its importance, it was demonstrated that STAT5 deficient murine mast cells cannot survive [17]. However, STAT5 appears to be important for IgE mediated mast cell activation as well [19]. Interestingly, STAT5 interacts with another pluripotent intracellular signalling molecule, termed phosphoinos-

itide 3-kinase (PI3K) [20]. PI3K is one of the most important molecules to be activated by G-protein coupled receptors such as the MRGPRX2 receptor, but is actually involved in many other intracellular processes to some extent [21]. These mechanisms have not yet been a target for treatment in mastocytosis, possibly because they have not been fully elucidated.

Figure 2

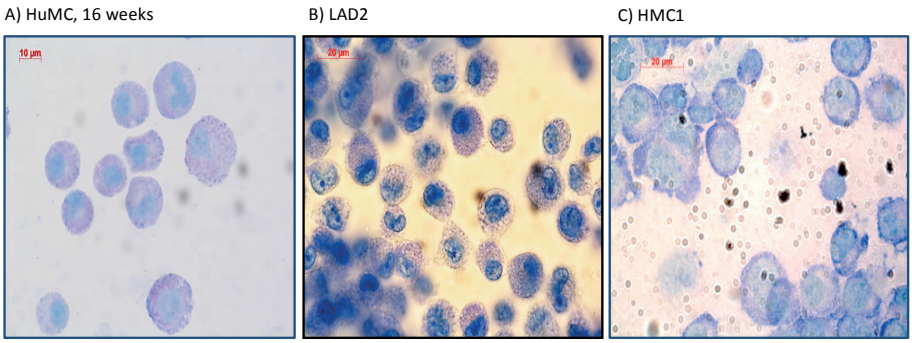


Figure 2. Toluidine blue staining. Shown are HuMC (A), LAD2 (B) and HMC1 (C) cells, 100x. HuMC and LAD2 show a typical granular pattern, whereas HMC1 cells are hypogranular.

In vitro research with mast cells is hampered by the slow proliferation rate of wild-type mast cells and the delicate nature of these cells. The isolation of mast cells from the human body is also difficult because they are tissue resident and not present in peripheral blood of healthy humans. Murine mast cells are often used, but have essential biological differences compared to human mast cells. Several human mast cell lines are available for researchers of mast cell biology (figure 2). LAD2 cells (Laboratory of Allergic Diseases type 2) are moderately resembling wild-type mast cells regarding their expression of the IgE receptor, their dependence on SCF for survival, and their slow doubling time of 2-3 weeks [22]. Human Mast Cell 1 (HMC1) was derived from a patient with mast cell leukemia and harbors 1 or 2 activating mutations in KIT, making them independent of SCF for survival [23]. These cells have a much more convenient doubling time of a few days, but have less similarities to wild-type mature human mast cells. For instance, they do not express the IgE receptor and release less histamine, tryptase and other typical mast cell me-

diators upon degranulation [23,24]. Lastly, HuMCs (human mast cells) are grown from CD34+ progenitors cells from peripheral blood or bone marrow [25]. These cells most closely resemble 'real-life' mast cells, but since it takes 12-16 weeks to grow them into mature mast cells, it is a time consuming and costly option.

Mast cells as immune regulators

As stated previously, mast cells are pluripotent cells and their functional variety enables them to communicate with many other cells via soluble mediators or direct (co-)stimulation [26]. As such, they are involved in various homeostatic processes and are considered important immune regulators. Depending on the route of activation, mast cells will produce pro-inflammatory cytokines and chemokines that can enhance both a type 1 as a type 2 immunological reaction [3,8]. Mast cells can also communicate with for instance dendritic cells and T-cells via extracellular vesicles as well as through direct cell-to-cell contact [27,28]. Non-immune cells that typically lie in proximity to mast cells such as keratinocytes, endothelial cells, nerve fibers and fibroblasts, can in their turn activate mast cells through the production of various soluble or cell-expressed signals [29,30]. An interesting cell type with regard to mast cell-immune interaction is the group 2 innate lymphoid cell (ILC2). Innate lymphoid cells are leukocytes that arise from lymphoid precursors and that lack classical T- or B-cell markers [31]. ILC2s are mostly present in skin, airway epithelium and mucosa. They can be found in peripheral blood but only in very low numbers. They are considered to be the native counterpart of T helper 2 cells, since they produce type 2 cytokines including IL-4, IL-5 and IL-13. Not surprisingly, ILC2s are implicated in allergic diseases such as asthma and atopic dermatitis [31]. An important route of activation occurs upon epithelial cell damage via thymic stromal lymphoprotein and IL-33, but also by lipid mediators that are produced by activated mast cells [31,32]. Considering these properties, the interaction between mast cells and ILC2s forms an interesting topic for research.

Mastocytosis

Mastocytosis is a rare hematological disease which is characterized by the accumulation of aberrant mast cells [33]. The World Health Organization recognizes different subtypes of mastocytosis. In cutaneous mastocytosis (CM), only the skin is involved [33]. This subtype is most common among children, and often spontaneously resolves in puberty [34]. It is yet unknown whether children who have persistent CM throughout adulthood have a similar prognosis as patients with systemic mastocytosis, and whether they have the same risk on developing complications such as anaphylaxis or osteoporosis. From one cohort study, it appeared that patients with CM did not have an increased risk of osteoporosis after correction for conventional risk factors for osteoporosis [35]. Among adults, the most common form of mastocytosis in the skin is urticaria pigmentosa, now called maculopapular cutaneous mastocytosis (MPCM) [36]. Adult-onset MPCM causes monomorphic livid-to-brown colored maculae, as depicted in figure 3. The aesthetic aspects of this, sometimes extensive, rash can be rather debilitating for patients. When MPCM originates after puberty, the chance of systemic involvement of mastocytosis is very high, even bordering on 100% in some studies [35,37]. When an adult patient with MPCM is not yet evaluated for systemic involvement, they are termed as having mastocytosis in the skin (MIS) and should be evaluated for the presence of systemic mastocytosis.

Systemic mastocytosis (SM) is defined by the World Health Organization (WHO) as the accumulation of neoplastic mast cells in at least one extra-cutaneous organ, most often the bone marrow (table 1) [33]. A bone marrow biopsy is thus virtually always necessary to confirm a clinical suspicion for SM. The most common form of SM is indolent systemic mastocytosis (ISM). This is a benign condition which has an excellent prognosis with regard to survival, but can be accompanied by a wide variety of bothersome symptoms. The prevalence of ISM in The Netherlands is approximately 13 in 100.000 residents [38].

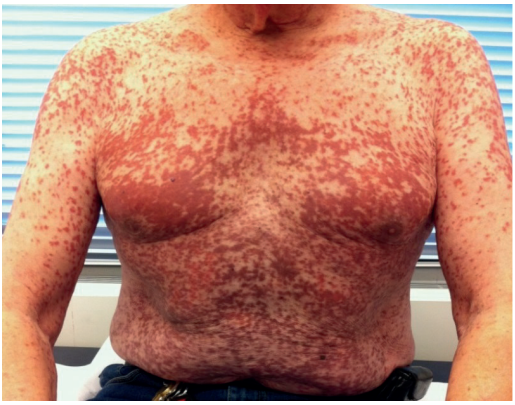


Figure 3.

A case of extensive maculopapular cutaneous mastocytosis.

Picture shown with consent from the patient.

In smoldering systemic mastocytosis (SSM), the patient has signs of a high mast cell load, defined as two or more B-findings (table 1) [33]. Although there is no organ failure in SSM, its survival is decreased compared to ISM, but this could partly be attributed to factors unrelated to mastocytosis such as age at diagnosis [39]. The term advanced systemic mastocytosis (AdvSM) describes the three most aggressive forms of mastocytosis: Aggressive systemic mastocytosis (ASM), systemic mastocytosis with an associated hematological neoplasm (SM-AHN), and mast cell leukemia (MCL). These subtypes have an unfavorable prognosis, although the perspectives for patients with ASM and MCL have been improved significantly since the advent of selective tyrosine kinase inhibitors such as midostaurin [40,41]. The prognosis of SM-AHN is largely defined by the associated hematological neoplasm.
The rest of this introduction only involves adult patients and systemic mastocytosis.

Pathophysiology of mastocytosis

SM is associated with an acquired mutation in the gene encoding for KIT, leading to uncontrolled proliferation and reduced apoptosis through autonomous stimulation of KIT, even in the absence of stem cell factor [42]. The D816V mutation is found in the majority of SM patients (80-90%), but other mutations in KIT have been described as well, mostly in children. This mutation is often limited to mast cells but can be found in other leukocytes too, mostly of the myeloid lineage [43]. The development of a highly sensitive method

to detect low levels of mutated KIT DNA by real time-quantitative polymerase-chain-reaction (RQ-PCR) has been a useful addition to the diagnostic arsenal for patients in whom SM is suspected. Although mast cells do not circulate in peripheral blood, a low level of D816V mutated KIT can be detected in most patients with SM. Probably, this reflects mast cell precursors that have left the bone marrow and are on their way to their place of homing. RQ-PCR also provides an quantitative allelic burden which has proven to be a measure for the total mast cell burden and can be used as a tool in the follow-up of patients with AdvSM in particular [43,44]. An allelic burden of over ~2% is considered to be an adverse prognostic sign, although this has not been confirmed prospectively [45].

Table 1. Diagnostic criteria for systemic mastocytosis as defined by the WHO [33]

Systemic mastocytosis			
Presence of 1 major and 1 minor criterium or 3 minor criteria. Major criterion: • Multifocal, dense MC infiltrate (with >15 MC per infiltrate) in bone marrow and/or extracutaneous organ. Minor criteria: • Presence of D816V KIT mutation in bone marrow, peripheral blood or extracutaneous tissue. • Expression of CD117 + either CD2 or CD25 in MC in bone marrow. • >25% atypical or spindle shaped MC. • Serum tryptase >20 µg/mL. *			
ISM	SSM	SM-AHN	ASM
Fulfills criteria for SM, without the presence of B- or C-findings ISMs- ISM without skin lesions ISMs+ ISM with skin lesions	Fulfills criteria for SM + ≥ 2 B-findings: • Hepato- and/or splenomegaly without organ dysfunction and/or lymphadenopathy • >30% MC infiltration in bone marrow and/or serum tryptase > 200 µg/mL • Signs of dysplasia or myeloproliferative disease without fulfilling criteria for SM-AHN	Fulfills criteria for SM + a non-mast cell lineage clonal hematological disease	Fulfills critera for SM + ≥1 C-finding: • Anemia, neutropenia of thrombocytopenia† • Hepatomegaly, ascites, hepatic dysfunction and/or portal hypertension • Malabsorption • Bone involvement with pathological fracture and/or osteolytical bone lesions • Splenomegaly with hypersplenism

MC: mast cells. SM: systemic mastocytosis. ISM: indolent systemic mastocytosis. SSM: smouldering systemic mastocytosis. ASM: aggressive systemic mastocytosis. SM-AHN: systemic mastocytosis with associated hematological non-mast cell lineage disease.

*Not valid in presence of an associated clonal hematological disease.
†Anemia Hb<10g/dL, neutropenia absolute neutrophil count <50x10^6, thrombopenia <1,500 x10^6.

Symptoms of mastocytosis and uncertainties in the clin ical care

Considering the wide armory of mast cells, it is not surprising that a surplus of these cells can also present with a diverse variety of signs and symptoms [46]. Symptoms of mastocytosis are either caused by the high levels of mast cell mediators, or by direct consequences of mast cell infiltration. Examples of mast cell mediator-related symptoms are itch, flushing, pyrosis, diarrhea, and osteoporosis. Moreover, between 30-49% of patients experience at least one episode of anaphylaxis in their life [47,48]. Anaphylaxis is defined as an acute, systemic and severe reaction secondary to mast cell activation. It can be life-threatening and logically causes substantial anxiety among patients [49]. Typically, anaphylaxis in patients with mastocytosis is not accompanied by urticaria or angioedema, and patients can experience a rapid decrease of blood pressure [50]. This rapid and profound circulatory shock might lead to life-threatening situations and several (near-)fatal cases of mastocytosis-related anaphylaxis have been described, fueling the fear of both patients and physicians [51]. The unpredictability of these symptoms are often labelled as one of the main factors that negatively influence the quality of life of patients with mastocytosis [52-54].

The most common trigger for anaphylaxis in mastocytosis is venom from Hymenoptera (mostly wasps) [47]. Conventional allergy tests such as intradermal tests or measurement of specific IgE to the culprit insect is frequently negative, which can complicate the management of these patients and makes it an unusable tool to predict future reactions to insect stings [55]. Next to Hymenoptera venom, patients with mastocytosis can have anaphylaxis to other venoms, for instance of the fire ant or jellyfish [56]. Other possible triggers for anaphylaxis are food, medications or physical stimuli such as temperature change, exercise or heat. Furthermore, an often feared elicitor is medication, with anesthetic agents, opiates, and radiocontrast media as the main culprit drugs [57]. This fear is based on pathophysiologic hypotheses and *in vitro* studies that showed mast cell activation upon opiates and radiocontrast media [58,59]. Several subsequently published case reports have caused the now common idea that these drugs pose a serious threat for patients with mastocyto-

sis. However, patients with mastocytosis relatively often need analgesic drugs because of nonspecific myalgia or pain due to osteoporotic fractures [60]. Moreover, the risk of cardiovascular morbidity is increased, rendering a frequent need for acetylsalicylic acid [61], and acetylsalicylic acid is a potent treatment for flushing related to mastocytosis. Lastly, prophylactic therapy with corticosteroids can cause side effects that are maybe unnecessary. It would thus be of great value to be able to predict whom is at risk for anaphylaxis and to which triggers.

A large amount of anaphylactic episodes actually remains idiopathic, even after evaluation by an allergist. As mentioned before, conventional allergy tests are unreliable in mastocytosis, possibly because in these patients anaphylaxis is often caused by direct mast cell activation for instance via the MRGPRX2 receptor, rather than activation via FcεR1. It is therefore very difficult to adequately consult a patient on their individual risk of anaphylaxis [50]. The uncertain risk of possible anaphylaxis has a negative influence on the quality of life of many patients [52,62]. Other factors that influence the disease-related quality of life are the cosmetic aspects of skin mastocytosis, cognitive problems, and bone pain [52,62]. Furthermore, at least half of the patients suffer from chronic fatigue [63]. The pathophysiological mechanisms that cause these symptoms have not been elucidated yet, but it is assumed that they are caused by the increased levels of pro-inflammatory cytokines [8,64]. Indeed, cohort studies in France showed an increased prevalence of depression and cognitive dysfunction among adults with mastocytosis [65,66]. However, they did not compare these data to adequate control groups. It thus remains unclear to what extent neuropsychiatric morbidity is specific to mastocytosis, or rather a consequence of having a chronic disease in general.

Management and follow-up

The treatment of mastocytosis depends on the subtype. Since patients with ISM have a normal life expectancy, cytoreductive treatment is often withheld because of concerns for disproportionate toxicity. However, ISM can be very

debilitating due to the aforementioned symptoms [52]. The goal of treatment in ISM is therefore symptom reduction, for which a cocktail of histamine receptor antagonists, leukotriene antagonists, and so-called mast cells stabilizers (e.g. cromoglycate) is often used [67]. High doses of these drugs are usually necessary to achieve adequate control of symptoms. As outlined before, the clinical picture of patients with mastocytosis is very heterogeneous. Because it is still not possible to predict the risk of anaphylaxis on an individual basis, it is recommended to distribute one or two adrenalin auto-injectors to each patient. If a patient has experienced prior Hymenoptera-associated anaphylaxis, venom subcutaneous immunotherapy might be indicated [68]. Of note, venom immunotherapy is not 100% effective in patients with mastocytosis, and needs to be continued lifelong [69].

A certain part of the patients with ISM has deblitating mast cell-mediator related symptoms that are refractory to high doses of anti-mediator medication. We currently cannot offer these patients much with regard to pharmacological therapy. Two studies have been published in which the efficacy of midostaurin and masitinib in ISM was investigated. For masitinib, only 18% of patients reached the primary endpoint of experiencing symptom relief in four domains [70]. Midostaurin was studied in a small non-controlled trial with highly symptomatic ISM patients and proved to have some degree of symptom relief in 75% [71]. Studies with other tyrosine kinase inhibitors that inhibit the function of KIT are currently running in patients with ISM, of which avapritinib seems to be quite promising based on the first results of phase 2 studies [72].

In advanced forms of SM, cytoreductive therapy might be indicated when organ failure is present or imminent [67]. Up to a few years ago, the treatment options for AdvSM were limited to corticosteroids, interferon-α, cladribin, or imatinib. These therapies were complicated by serious side effects and often not very effective [73]. Allogenic hematopoietic stem cell transplantation has had varying results and considerable mortality and is mainly advised for SM-AHN or acute MCL [74]. Since 2017, the tyrosine kinase inhibitor midostaurin was approved for the treatment of AdvSM in both the USA and Europe. This

has led to an improvement in survival rates of particularly MCL. However, midostaurin has considerable side effects, with over 80% of patients experiencing moderate to severe nausea [75]. Furthermore, the neoplastic cells can become resistant to its effects over time. Although promising new tyrosine kinase inhibitors are currently studied, there is still a need to broaden the therapeutic arsenal for the treatment of all subtypes of mastocytosis.

1.2 Aims of this thesis

The aim of this thesis was to address clinical questions on mastocytosis from a practice-based perspective. The first project was a cohort study of all adult patients in the Erasmus MC up to that time. The findings of this study led to a series of research questions and hypotheses that were studied in the rest of this thesis. Since it is a rare and miscellaneous disease, a substantial part of daily clinical practice is based on expert opinion and theoretical hypotheses instead of on scientific evidence. This thesis was aimed at finding scientific grounds for some of those "myths".

Firstly, several studies have previously demonstrated an increased prevalence of depression and anxiety among patients with mastocytosis, but none of these studies made a comparison with healthy controls or people with other chronic diseases. We hypothesized that the increased prevalence of psychological symptoms in mastocytosis could at least partially be explained by the impact of having a chronic disease in general. We therefore conducted a cross-sectional study using questionnaires for psychological symptoms and health-related quality of life and compared these results to several norm groups.

Furthermore, several diagnostic issues were investigated. Serum tryptase is used as a screening tool for SM and the WHO criterion of ≥20 μg/L is often seen as the cut-off value for that purpose. However, we suspected that this seemingly arbitrary cut-off level of 20 μg/L has a low sensitivity. We performed a retrospective study that described the serum tryptase levels of patients with SM at the moment of diagnosis. Also, we studied whether ultrasonography of the abdomen is useful in the management and follow-up of patients with SM.

This study also provided information on the prognosis of ISM in general. Lastly, we tried to find a method to predict Hymenoptera-related anaphylaxis in patients with mastocytosis, by using the basophil activation test.

Another part of this thesis involves iatrogenic anaphylaxis in patient with mastocytosis. The main aim here was to find scientific rationale to generate advices on the administration of medication to patients with mastocytosis. In daily practice, we found that most patients could tolerate NSAIDs and other drugs that are deemed dangerous for patients with mastocytosis. To scientifically corroborate this, we performed a randomized controlled trial in which patients with SM were challenged with acetylsalicylic acid. Furthermore, we reviewed the current literature on perioperative management and formulated practical recommendations for prophylaxis prior to the administration of radiocontrast media and anesthesia.

The last chapter in this thesis contains translational research. There is still a lack of effective pharmacological therapies available to treat mast cell mediator related symptoms. Based on the effects on itch in patients with myeloproliferative neoplasms, we postulated that inhibitors of the JAK-STAT pathway would probably be effective to inhibit mast cell activation. This was studied in vitro.

Lastly, although mastocytosis is a disease of mast cells primarily, those aberrant mast cells might theoretically influence other immune cells in many ways. Since ILC2s are one of a few human cell types that can express KIT in their mature form, we were interested in the ILC2 numbers and phenotype in patients with systemic mastocytosis. This was investigated in the last part of this thesis.

Chapter 2

Mastocytosis is a multifaceted disease

2.1 Systemic mastocytosis: a cohort study on clinical characteristics of 136 patients in a large tertiary centre.

M.A.W. Hermans, M.J.A. Rietveld, J.A.M. van Laar, V.A.S.H. Dalm, M. Verburg, S.G.M.A. Pasmans, R. Gerth van Wijk, P.M. van Hagen, P.L.A. van Daele

European Journal of Internal Medicine 2016 May;30:25-30

Abstract

Background: Systemic Mastocytosis (SM) is a rare heterogeneous disease which is characterized by the aberrant proliferation of mast cells. It can be divided in various subtypes with different phenotypes and prognosis. Here, we report on the clinical characteristics of 136 SM patients.

Methods: A retrospective cohort study was conducted from January 2009 to September 2014 in a large tertiary center in the Netherlands. We included all patients who fulfilled WHO criteria for SM. Data were collected from electronic patient files.

Results: 124 patients had indolent SM (ISM) (91.2%), 7 had aggressive SM (ASM) (5.1%) and 5 had SM with associated haematological non-mast cell lineage disease (SM-AHNMD) (3.7%). There was no progression from ISM to advanced SM subtypes, but 1 patient with ASM developed chronic myelocytic leukemia, 2 years after diagnosis. The average time to diagnosis for the whole population was 8,1 years (range 0-49 years). The most frequent triggers for work-up- skin involvement, anaphylaxis and osteoporosis- were characterized by an interval to diagnosis of 10.9, 2.9 and 7.5 years, respectively. 32 patients (23.5%) had a serum tryptase level below the cut-off value of 20 ng/ml at the time of diagnosis, but these patients did not have significant differences in clinical phenotype.

Conclusions: SM comprises a wide spectrum of signs and symptoms and its often atypical presentation can delay the establishment of the diagnosis substantially. Skin involvement, anaphylaxis and unexplained osteoporosis should trigger analysis for mastocytosis. A normal serum tryptase does not exclude the diagnosis of SM.

Introduction

Mastocytosis is a rare systemic disease which is characterized by uncontrolled proliferation of aberrant mast cells.[1] According to the definition of the World Health Organization (WHO) it is a myeloproliferative disease with different subtypes.[76] In systemic mastocytosis (SM), at least one extracutaneous organ is affected. Systemic mastocytosis is divided in various subtypes (table 1). Most patients have indolent SM (ISM), which generally has a mild course and does not affect overall survival. It is increasingly recognized that ISM patients with or without skin lesions (ISMs+ or ISMs-, respectively) have clinically distinct phenotypes.[77] Smouldering SM (SSM) is a relatively new subtype of ISM and is defined by the presence of organ involvement without organ dysfunction. In SM with associated hematological non-mast cell lineage disease (SM-AHNMD), the prognosis is determined by the associated condition. Furthermore, aggressive SM (ASM) is characterized by organ dysfunction due to infiltration of mast cells. This subtype often needs more intensive treatment with cytoreductive therapy.

Table 1. Diagnostic criteria for systemic mastocytosis

Systemic mastocytosis			
Presence of 1 major and 1 minor criterium or 3 minor criteria. Major criterium: • Multifocal, dense MC infiltrate (with >15 MC per infiltrate) in bone marrow and/or extracutaneous organ. Minor criteria: • Presence of D816V KIT mutation in bone marrow, peripheral blood or extracutaneous tissue. • Serum tryptase >20 µg/mL. • Expression of CD117 + either CD2 or CD25 in MC in bone marrow. • >25% atypical or spindle shaped MC.			
ISM	**SSM**	**SM-AHNMD**	**ASM**
Fulfills criteria for SM, without the presence of B- or C-findings ISMs- ISM without skin lesions ISMs+ ISM with skin lesions	Fulfills criteria for SM + ≥ 2 B-findings: • Hepato- or splenomegaly without organ dysfuntion. • Lymphadenopathy. • >30% MC infiltration in bone marrow. • Serum tryptase > 200 ng/mL. • Signs of dysplasia or myeloproliferative disease without fulfilling criteria for SM-AHNMD.	Fulfills criteria for SM + a non-mast cell lineage clonal hematological disease (myelodysplastic syndrome, acute myeloid leukemia, non-Hodgkin lymphoma or myeloproliferative neoplasm).	Fulfills critera for SM + ≥1 "C-findings": • Anemia, neutropenia of thrombocytopenia* • Hepatomegaly, ascites, hepatic dysfunction and/or portal hypertension. • Malabsorption. • Bone involvement with pathological fracture and/or osteolytical bone lesions. • Splenomegaly with hypersplenism.

MC: mast cells. SM: systemic mastocytosis. ISM: indolent systemic mastocytosis. SSM: smouldering systemic mastocytosis. ASM: aggressive systemic mastocytosis. SM-AHNMD: systemic mastocytosis with associated hematological non-mast cell lineage disease.

*Anemia Hb<10g/dL, neutropenia absolute neutrophile count <50x10^6, thrombopenia <1,500 x10^6

The prevalence of SM in The Netherlands is approximately 13 in 100.000 residents, which appears to be in accordance with other European countries. [38] In 80-90% of patients, the D816V mutation is found in the gene encoding for c-KIT, a tyrosine kinase that functions as stem cell factor (SCF) receptor. A small proportion of patients has other mutations in c-KIT. This mutation leads to uncontrolled proliferation and inhibition of apoptosis through continuous stimulation of c-KIT, even in the absence of SCF. [42] However, not every SM patient has the D816V mutation, and conversely, not all patients with the D816V mutation express the same phenotype. Therefore, other unknown factors also have to play a role in the pathogenesis of SM.[4]

SM is known to cause a wide diversity of symptoms. These can vary from the typical urticaria pigmentosa or flushing and itching, to less specific symptoms like osteoporosis, diarrhea or unexplained syncope. Most symptoms are caused by high levels of mast cell mediators, mainly histamine and proinflammatory cytokines. [78] Due to this heterogeneous presentation, diagnosing this disease, particularly ISM, can be a true challenge which requires high clinical suspicion. It does not need further explanation that delay in the diagnostic process can lead to several undesired consequences like organ dysfunction, life threatening anaphylaxis or severe osteoporosis. [52,79]

The objective of this study is to describe the clinical characteristics of all patients with SM who were referred to the Erasmus University Medical Centre in the last 5 years. Hereby, we hope to create a better understanding of the way patients present to us and hopefully improve the diagnostic process.

Methods

Patient selection and follow-up

We selected all patients who visited the Erasmus MC University Medical Centre from January 2009 to September 2014 and fulfilled the WHO criteria for systemic mastocytosis.[76] Recently, the NFU (Dutch federation of academic medical centres) classified the Erasmus MC Mastocytosis Centre as a centre of excellence. Included patients could have visited either the outpatient clinic of clinical immunology, hematology or allergology. Therefore, no uniform diagnostic

work-up was performed. Also, guidelines on the work-up of SM have changed in the studied period. However, general laboratory tests including serum tryptase and bone marrow examination was performed in most patients. Patients were routinely seen once yearly for follow-up purposes in the outpatient clinic. Yearly, data on clinical symptoms, laboratory markers (including serum tryptase) and an abdominal ultrasound were performed to screen for progression. Once in every 2 years, bone densitometry was performed for follow-up on osteoporosis. By retrospectively studying the patient files, we collected a wide array of data: the symptom that triggered referral and analysis, the time of diagnosis, other mastocytosis related symptoms, various laboratory results and organ involvement. Most patient were followed-up in our centre. For the patients who were not followed-up in our centre, we only retrieved information on their visits in our centre and on their survival until September 2014.

Definitions

For the different subtypes of SM, we used the WHO classification.[76] For skin involvement, both urticaria pigmentosa and teleangiëctasia macularis eruptiva perstans were accounted for. Neuropsychiatric symptoms were defined as every psychiatric diagnosis in the patient's medical history or the current use of psychiatric medication. Cytopenia involved anemia (Hb <10 g/dL), leukopenia (<400x 10^6) and/or thrombocytopenia (<1,500x 10^6). Osteoporosis was classified according to T-scores: osteoporosis = T-score $\leq$ - 2.5; osteopenia = T-score -1 to -2.5; osteosclerosis = T-score $\geq$ + 2.5. A pathological fracture was defined as a spontaneous fracture directly linked to SM. Osteoporotic vertebral fractures were not included in this definition.

Statistical analysis

We used IBM Statistics SPSS 21 for all analyses. Frequencies, percentages with range or standard deviation were calculated for all variables. The subtypes of SM were compared with a one-way ANOVA test for continuous variables and with a chi-square test for dichotomous variables. Correlation coefficients were calculated with Spearman's rho.

Mutational analysis

In brief, 400 µL of EDTA-preserved bone marrow was used to isolate DNA with the MagNApure (Roche Molecular Systems, Mannheim, Germany) according to the manufacturer's instructions. Subsequently, a standard solution of 50 ng/µL was prepared using the Nandrop (Shimadzu Corporation, Kyoto, Japan) of which 3 µL was used for each analysis. Detection of D816V KIT mutation was performed by two independent methods in all samples: the LightCycler System (Roche Molecular Systems, Mannheim, Germany) for the primary result and the Taqman System (Fisher Scientific, Amsterdam, The Netherlands) for confirmation. In the LightCycler assay, the amplification rate of mutated DNA over wildtype DNA was improved by adding Locked Nucleic Acid (LNA, Tib Molbiol, Berlin, Germany). The mutation was detected by melting point analysis. In the Taqman assay, a mismatched positive primer with a much higher affinity for the mutant DNA compared to wildtype DNA was used. A cycle time (CT) value <40 was considered as positive for the presence of D816V. In both assays the detection limit is 0.1% mutated copies. Two negative controls (a blanc without DNA and one with wildtype DNA) and two positive controls (one with diluted plasmid DNA of the mutation to assess the detection limit and one with the D816V mutation) were used. The wildtype and mutated controls were left-over samples of which the genotype was established in a previous run.

Results

The total patient population consisted of 136 persons of whom 58 (42.6%) were female. The mean age at diagnosis was 48.5 years (range 14-80). The mean total duration of illness from the start of symptoms until the moment of inclusion was 15.9 years (range 0-71). At inclusion in September 2014, 4 patients had died (2.9%), of whom 3 patients had SM-AHNMD and one had ASM. Unfortunately, the cause of death was only available of 1 patient:, she died due to substantial organ infiltration by ASM. The mean duration of illness of the deceased patients was 14.3 years (range 6-25). The most common presenting symptoms were skin involvement and anaphylaxis (table 2). The aver-

age time from the start of symptoms to diagnosis was 8.1 years (range 0-49). When divided by symptom, the mean time to diagnosis was longest for skin involvement (10.9 years) and shortest for flushing (1.3 years).

Table 2. Symptom that triggered diagnostic work-up.

Presenting symptom	N=136 n (%)	Time to diagnosis mean (SD)
Skin involvement	68 (50)	10,9 (10,1)
Anaphylaxis/angioedema	34 (25)	2,9 (6,2)
Osteoporosis/bone pain	10 (7,4)	7,5 (6,3)
Fatigue	8 (5,9)	6 (8,8)
Diarrhea	4 (2,9)	12 (9,4)
Syncope	4 (2,9)	8,3 (8,5)
Flushing	4 (2,9)	1,3 (1,9)
Miscellaneous*	4 (2,9)	

*Miscellaneous: leukopenia (1), accidental finding with biopsy (2), itching (1).

Vitamin B12

For the whole population, vitamin B12 levels tended to correlate with serum tryptase levels (rho = 0.427; p=0.060) and were significantly correlated with eosinophilia (rho= 0.491, p=0.028). Of all 10 patients in whom eosinophilia was present, it was always mild to moderate with a mean absolute eosinophil count of $750x10^6$ (range 400 – 1.730). Hypereosinophilic syndrome and chronic eosinophilic leukemia were ruled out in these patients by bone marrow biopsy and molecular studies.

Anaphylaxis

When taking the whole population into account, 42 patients experienced at least one anaphylactic reaction. 24 cases were triggered by insect venom of which 21 were Hymenoptera (87.5%). In 3 patients, anaphylaxis was caused by medication: NSAID or radiocontrast media. Another 4 reactions were related to food (peanut or shrimp) and in 11 patients no trigger could be identified. A specific IgE level was determined in 31 patients and was detectable in 12 patients (38.7%) from which 11 cases were IgE against Hymenoptera venom.

Serum tryptase levels

Interestingly, 32 patients (23.5% of the total population) had a serum tryptase level <20 µg/l at diagnosis and 9 of these even had a serum tryptase level <11.4 µg/l. 22 of the 32 patients never reached elevated tryptase levels during follow-up. When dividing the study population according to serum tryptase level in three groups: ≤11.4 µg/l; 11.4-20 µg/l; ≥20 µg/l, there was no significant difference in age, D816V status, nor in their clinical phenotype (data not shown). One exception was bone disease: although not reaching statistical significance, patients with a serum tryptase level >20 µg/l showed a trend towards having more osteoporosis (35.4% vs 60.8% respectively; p 0.091) and more often had pathological fractures (0% vs 4.9%; p 0.026) than patients with a serum tryptase level <20 µg/l.

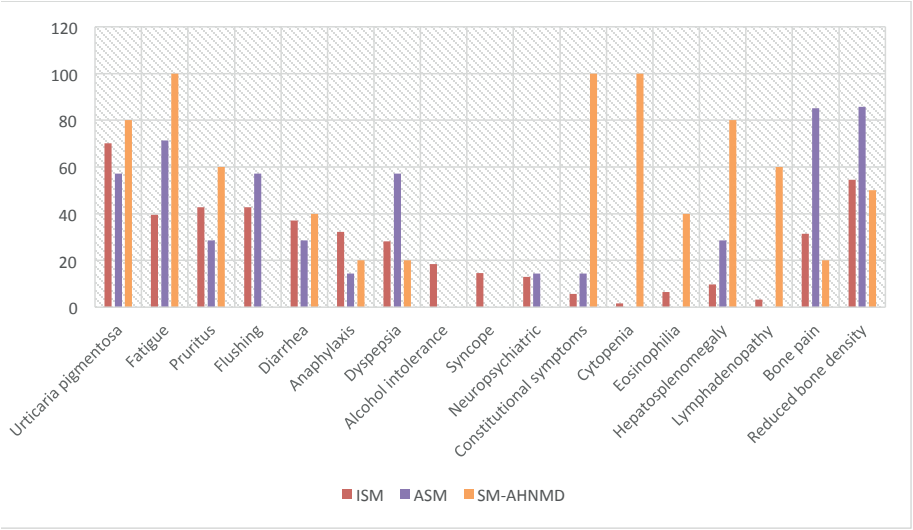
Neuropsychiatric symptoms

In our population, 17 patients (12.5%) had neuropsychiatric symptoms. 7 of those suffered from depression, 5 had cognitive problems, 3 patients had an anxiety disorder. Furthermore, 1 person had schizophrenia and 1 had Attention Deficit Hyperactivity Disorder. The cognitive problems mainly consisted of lack of concentration and decreased short-term memory.

Bone density abnormalities

We had access to bone density measurements of 129 patients. Of those, 29 (22.4%) had osteoporosis and 41 (31.8%) had osteopenia. The average age of the patients with abnormal bone density was 52 years (range 23-72) and almost half of them was male (48%). Osteosclerosis was only found in 3 persons. Bone pain was reported by 46 patients (33.8% of the total population) but in 6 of 7 ASM patients (85.7%).

Figure 1. Symptoms associated with ISM, ASM and SM-AHN



Classification

According to WHO criteria, 124 of 136 patients had ISM (91.2%), 7 had ASM (5.1%) and 5 had SM-AHNMD (3.7%). Of all patients with SM-AHNMD, 3 had chronic myelomonocytic leukemia, 1 had acute myeloid leukemia AML-M5 and 1 had myelodysplastic syndrome. C-findings that classified the 7 patients as ASM were mostly bone-related; 5 patients had pathological fractures, 1 of whom also had osteolytical lesions. The other 2 ASM patients had malabsorption, and hepatosplenomegaly with portal hypertension and ascites, respectively. We did not record any progression from ISM to another subtype during follow-up, however, 1 patient with ASM developed chronic myelocytic leukemia (and thus SM-AHNMD) 2 years after diagnosis.

In patients with ISM, skin involvement was the most common symptom, most often urticaria pigmentosa. Furthermore, they mainly suffered from classical mast cell-mediator related symptoms like itching, flushing and diarrhea (figure 1). The differences between ISMs- and ISMs+ patients are outlined elsewhere in this article. Of all patients with ASM, 6 out of 7 had osteoporosis and

5 patients had at least once suffered a pathological fracture. Fatigue and bone pain also were significantly more prevalent in this group when compared to ISM (table 3). For SM-AHNMD, all patients had cytopenias and constitutional symptoms at diagnosis. Moreover, erythrocyte sedimentation rate and vitamin B12 levels were significantly higher than in other groups.

Indolent systemic mastocytosis without skin lesions

Patients with ISM were further divided into patients with and without skin involvement (ISMs- or ISMs+, respectively). Of ISMs- patients, 57% was male versus 34% of ISMs+ patients. ISMs- patients more often had anaphylaxis but less often experienced mast cell mediator related symptoms (table 3). In fact, anaphylaxis was the presenting symptom for 60% of ISMs- patients, compared with 14% of ISMs+ patients. For the latter, skin lesions were the most common presenting symptom (72%). The trigger for anaphylaxis in ISMs- patients was Hymenoptera venom in 64%, food in 14% and was unknown in 23%. For ISMs+ patients, anaphylaxis was triggered by Hymenoptera venom in 39%, food or NSAID in 17% and the trigger was unknown in 44%. Serum tryptase at baseline was lower in ISMs- patients compared with ISMs+ patients (mean 34.5 vs. 65.6 µg/l, p 0.037). However, the number of patients with a serum tryptase level <20 µg/l was similar in ISMs- and ISMs+ patients (27% vs. 25%, p 0.723) and the same accounted for serum tryptase levels below the 'normal' cut-off value of <11.4 µg/l (5% vs. 8%, p 0.617).

Table 3. Clinical and laboratory characteristics.

Characteristic*	All patients	ISMs- (n=37)	ISMs+ (n=87)	ASM (n=7)	SM-AHNMD (n=5)	p†
Age at diagnosis in years	48.5 (14-80)	51.8 (30-76)	46.1 (14-75)	52.6 (32-68)	60.4 (43-80)	0.010
Total duration of illness in years	15.9 (0-71)	13.0 (0-31)	17.1 (2-71)	17.8 (4-46)	13.4 (6-25)	NS
Time to diagnosis in years	8.1 (0-49)	5.7 (0-24)	9.5 (0-49)	7.5 (1-28)	1.6 (0-5)	NS
ESR (mm/hour)‡	11.2 (1-105)	10.4 (2-32)	8.4 (1-33)	10 (2-29)	64 (25-105)	0.000
Vitamin B12 (ng/l) ‡	535.7 (118-1475)	353.7 (148-622)	458 (212-1061)	312.5 (118-507)	1210.7 (826-1475)	0.002
WHO criteria§						
MC infiltrates in bone marrow on histologic evaluation	124/126 (98.4)	22/25 (88)	67/73 (91.8)	7/7 (100)	4/5 (80)	NS
Flow cytometry positive	84/89 (94.4)	26/27 (96.3)	52/55 (94.5)	3/4 (75)	3/3 (100)	NS
D816V mutation	63/78 (80.8)	14/23 (60.9)	44/49 (89.8)	2/3 (66.7)	3/3 (100)	0.023
Tryptase (µg/l)*	61 (4.2-457)	34.5 (8-103)	65.6 (4.2-457)	61.5 (21.2-99.3)	206 (72.8-280)	0.000
Aberrant MC morphology in bone marrow smear	110/120 (91.7)	32/35 (91.4)	67/73 (91.8)	7/7 (100)	4/5 (80)	NS
Symptoms	**All patients**	**ISMs- (n=37)**	**ISMs+ (n=87)**	**ASM (n=7)**	**SM-AHNMD (n=5)**	**p†**
Skin involvement	95/136 (69.9)	0/37 (0)	87/87 (100)	4/7 (57.1)	4/5 (80)	0.000
Fatigue	59/136 (43.4)	13/37 (35.1)	36/87 (41.4)	5/7 (71.4)	5/5 (100)	0.019
Itching	58/136 (42.6)	5/37 (13.5)	48/87 (55.2)	2/7 (28.6)	3/5 (60)	0.000
Flushing	57/136 (41.9)	9/37 (24.3)	44/87 (50.6)	4/7 (57.1)	0/5 (0)	0.009
Diarrhea	50/136 (36.8)	3/37 (8.1)	43/87 (49.4)	2/7 (28.6)	2/5 (40)	0.000
Anaphylaxis	42/136 (30.9)	22/37 (59.5)	18/87 (20.7)	1/7 (14.3)	1/5 (20)	0.000
Dyspepsia	40/136 (29.4)	7/37 (18.9)	28/87 (32.2)	4/7 (57.1)	1/5 (20)	NS
Alcohol intolerance	18/105(13.2)	4/37 (10.8)	14/68 (20.5)	0/3 (0)	0/4 (0)	NS
Syncope	18/136 (13.2)	8/37 (21.6)	10/87 (11.5)	0/7 (0)	0/5 (0)	NS
Neuropsychiatric	17/136 (12.5)	4/37 (10.8)	12/87 (13.8)	1/7 (14.3)	0/5 (0)	NS
Constitutional symptoms	13/136 (9.6)	2/37 (5.4)	5/87 (5.7)	1/7 (14.3)	5/5 (100)	0.000
Eosinophilia	10/135 (7.4)	1/37 (2.7)	7/87 (8)	0/7 (0)	2/5 (40)	0.023
Cytopenia	7/135 (5.2)	2/37 (5.4)	1/87 (1)	0/7 (0)	5/5 (100)	0.000
B-findings						
Hepatomegaly	18/135 (13.2)	1/37 (2.7)	11/87 (12.6)	2/7 (28.6)	4/5 (80)	0.000
Splenomegaly	16/134 (11.9)	1/37 (2.7)	3/87 (3.4)	1/7 (14.3)	4/5 (80)	0.000
Serum tryptase >200 ng/mL	9/136 (6.6)	0/37 (0)	6/87 (6.9)	0/7 (0)	3/5 (60)	0.000
>30% MC in bone marrow	2/33 (6.1)	0/31 (0)	1/21 (4.8)	1/3 (33.3)	0/3 (0)	NS
Lymphadenopathy	7/136 (5.1)	1/37 (2.7)	3/87 (3.4)	0/7 (0)	3/5 (60)	0.000

Abbrevations: ISMs-: Indolent systemic mastocytosis without skin lesions, ISMs+: Indolent systemic mastocytosis with skin lesions, ASM: aggressive systemic mastocytosis, SM-AHNMD: systemic mastocytosis with associated hematologic non-mastcell disease, NS: not significant, ESR: erythrocyte sedimentation rate, MC: mast cells.
*Values as mean + range.
† p value for the 4 subtypes compared.
‡ ESR was tested in 103 patients, vitamin B12 level was tested in 20 patients.
§ Each patient could have more than one symptom. Values as n(%) except for mean tryptase levels.

Treatment

The majority of patients was treated symptomatically with histamine antagonists and cromoglycate; respectively 93 (70%) and 17 (12.7%). Furthermore, 10 patients received glucocorticoids, but mostly as an adjunct to cytoreductive therapy. When cytoreductive therapy was indicated, imatinib was most often used. This is partly due to the fact that a study on imatinib was conduct-

ed during part of the studied period.[80] Imatinib was prescribed to 9 patients with ISM, 1 with ASM and 3 patients with SM-AHNMD. Of the ISM patients who were treated with imatinib, indication for treatment was disabling itching, splenomegaly, or gastrointestinal complaints. It was effective in 5 patients in total and mainly seemed to improve fatigue, skin lesions and serum tryptase levels. For further information on imatinib treatment, we refer to the article of Droogendijk et al., which included our patients.[80] The second most used cytoreductive agent was cladribin in 4 patients, of whom 3 suffered from SM-AHNMD and 1 had ISM. Cladribin was effective in 1 patient who had SM-AHNMD; it mainly improved gastrointestinal symptoms and reduced pancytopenia. Interferon was applied in 1 patient, but was discontinued early because of adverse effects. A last notable fact is that only 62 patients (46.3%) were equipped with an epinephrine autoinjector. Of all patients who initially presented with anaphylaxis, 31 (73.8%) had an epinephrine autoinjector.

Discussion

In this study, we describe a relatively large population of SM patients in a tertiary center. The clinical presentation of our patients was rather diverse and mainly included skin involvement and mast cell mediator-related symptoms. We found a considerable diagnostic delay. The mean time to diagnosis was 8.1 years. Until now, only one other study investigated the time to diagnosis and found a median of 33 months (range 0-516 months). [40] The diagnostic process was more delayed when patients had atypical symptoms. However, even when anaphylaxis was the first symptom, the mean time to diagnosis was still 2.9 years. This substantial delay might have various reasons, but an important explanation might be the lack of clinical suspicion with many physicians. Moreover, there could have been patient delay because patients with (asymptomatic) skin involvement often postpone seeking medical advice.

Our study encompasses the care of SM patients from 2009 to 2014. During this period, a lot was learned on this disease and our study shows the evolution of care for SM patients in the last years. For instance, standard mutational

analysis only included D816V c-KIT mutation, which might have led to misdiagnosing of a few patients who did not have the D816V mutation, but another mutation in the gene encoding for c-KIT. Our data again underline the need for standardized algorithms for work-up and treatment, which has been demonstrated before in work from the Spanish and European Networks on Mastocytosis.[81,82] Fortunately, since recent years, we are following a standardized diagnostic workflow for SM patients in our center.

Another important finding of our study is the high rate of osteoporosis and osteopenia. These figures are comparable to other Western European literature on SM. [77,83,84] Pathological fractures were rare. However, we did not account for so-called fragility fractures like vertebral fractures because they are not included in the diagnostic criteria for Systemic Mastocytosis. Obviously, vertebral fractures can also cause significant disability. The patients with osteoporosis in our population were not the typical elderly lady, on the contrary: half of them was middle-aged and/or male. This is in line with other literature on this topic and leads to the recommendation that SM needs to be considered in (male) patients without conventional risk factors for osteoporosis.[85]

In 17 patients (12.5%), neuropsychiatric morbidity was reported. When evaluating neuropsychiatric symptoms in SM patients, data strongly vary. The prevalence seems to be around 21-24% in other general population studies [48,83,86] However, when neuropsychiatric symptoms are specifically studied with validated questionnaires, much more impressive results are shown: 74% of SM patients reports subjective cognitive problems [65] and 56% fulfills the criteria for depression. [66] We therefore suspect that neuropsychiatric symptoms are not sufficiently recognized. In line with our previous remark on the importance of standardized work-up methods, implementing a standardized questionnaire in routine outpatient visits might improve the recognition and treatment of these symptoms as well.

Similar to other studies [40,83], there were clear differences in clinical symptoms between the subtypes in our population. Most differences are inherent to the diagnostic criteria. Remarkably, patients with SM-AHNMD had significantly higher serum tryptase and vitamin B12 levels compared with the other two subtypes. A spontaneously elevated vitamin B12 level has mainly been described with hematological malignancies and hypereosinophilic syndrome, where it is caused by a raised serum haptocorrin level, which is a transportation protein for vitamin B12. [87] In hypereosinophilic syndrome, vitamin B12 and serum tryptase levels seem to be positively correlated to each other. [88] We were able to confirm this correlation in our patients. Unfortunately, data on vitamin B12 levels were available in a limited number of patients which only leaves us to speculate on the clinical significance of this association.

A relatively new subdivision of ISM patients in those with and without skin lesions was also made in our cohort. ISMs- patients were more often male, more often had anaphylaxis of which wasp venom the most common trigger, and reported less mast cell mediator related symptoms. These characteristics are in accordance with other cohort studies on this topic.[77] Data on serum tryptase levels in ISMs- patients are contradictory. One Dutch study postulated that in patients without skin lesions/UP, a normal serum tryptase level sufficiently excluded SM.[15] However, in our cohort, a considerable number of ISMs- patients had serum tryptase levels below the normal cut-off value and over a quarter of ISMs- patients had a baseline serum tryptase of <20 µg/l.

A notable difference to other studies is the favorable prognosis in our cohort. In another large cohort studies of SM, the median survival was 41 months for ASM patients and 24 months for SM-AHNMD patients.[40] Of course, we had small numbers of patients with ASM or SM-AHNMD. Also, most patients with ASM classified for this subtype due to bone lesions which generally do not influence one's survival. Moreover, in recent years, the treatment for non-mast cell hematological diseases in general has greatly improved which could explain part of the survival of our SM-AHNMD patients.

Several limitations of this study should be addressed. Firstly, the retrospective design could lead to incomplete data, which for instance explains the relatively low prevalence of neuropsychiatric symptoms in our population. Secondly, we searched for eligible patients via diagnostic codes in the electronic patient file. This method inevitably excludes patients who have received an inaccurate diagnostic code. Lastly, we did not differentiate smouldering SM (SSM) from ISM. This is due to the fact that we did not work with the separate diagnosis of SSM in our daily practice at that time. However, we provided information on B-findings in table 3 to give an impression of how many patients actually had SSM.

Conclusion

Here, we present a relatively large population of 136 SM patients who visited a tertiary center over a period of 15 years. This study shows the considerable diagnostic delay that is partly due to the heterogeneous nature of this disease. Symptoms are often present for years before the diagnosis is made. The diagnostic delay is generally shorter in patients with more classical or more severe symptoms. A high clinical suspicion is needed for prompt diagnosis. Moreover, our retrospective study underlines the importance of standardized methods for work-up and treatment.

Importantly, not all patients with SM have raised serum tryptase levels at diagnosis and clinicians sometimes need to be triggered by other small clues like spontaneously raised vitamin B12 levels or unexplained osteoporosis. However, SM also needs to be included in the differential diagnosis for patients with (recurrent) anaphylaxis or long-lasting abdominal complaints. Finally, points of interest in the care for SM patients are the prescription of epinephrine autoinjectors and recognition of neuropsychiatric symptoms.

2.2 Psychological functioning and quality of life in patients with mastocytosis: a cross-sectional study.

M.R. Vermeiren, L.W. Kranenburg, P.L.A. van Daele, R. Gerth van Wijk, M.A.W. Hermans

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Abstract

Background: Psychological symptoms appear to be frequent among patients with mastocytosis and can significantly affect their quality of life. Yet, it remains unclear whether and to which extent this may be the case. We aimed to investigate both the presence and type of psychological symptoms and quality of life in patients with mastocytosis.

Methods: A cross-sectional cohort study in adult patients with mastocytosis. All patients completed the 36-item Short Form health survey (SF-36) and the 90-item Symptom Checklist (SCL-90). Results were compared to established norm groups of patients with other diseases (chronic pain and cancer) and healthy populations.

Results: Fifty patients were included. Seventy percent had indolent systemic mastocytosis. Patients with mastocytosis scored significantly worse than a healthy norm group on the total SCL-90 and more specifically on the dimensions of *depression*, *somatization*, *sleeping problems* and *inadequacy of acting and thinking*. Strikingly, patients with mastocytosis scored similar to cancer patients on the SF-36 subdomains of *general health perception* and *vitality*. There was however no significant difference in the *mental health* subdomain of the SF-36 when compared with a healthy norm group.

Conclusion: In addition to the presence of psychological symptoms, the physical symptoms that are associated with mastocytosis also have a significant impact on the quality of life of patients with this disease. Therefore, correct treatment through symptom control and psychological counselling is necessary.

Introduction

Mastocytosis is a chronic hematological disease that is characterized by an accumulation of aberrant mast cells in one or several organs. Symptoms can be caused by tissue infiltration of mast cells or by the excessive release of mast cell mediators such as histamine, eicosanoids, heparin and many different cytokines.[89] In cutaneous mastocytosis, only the skin is involved, whereas systemic mastocytosis is characterized by the involvement of at least one extracutaneous organ, most often the bone marrow.[33] The WHO has formulated several subtypes of systemic mastocytosis (SM), ranging from indolent to more aggressive forms. When a patient has cutaneous signs of mastocytosis but a bone marrow examination has not been performed, they are termed to have mastocytosis in the skin (MIS).[90] Indolent systemic mastocytosis (ISM) is the most common subtype of SM and has virtually no influence on life expectancy.[40] Nevertheless, it is associated with significant disability – meaning: one or more symptom(s) that prevent the patient from continuing their everyday life. The clinical presentation is very heterogenous and there are various symptoms that negatively impact the quality of life of these patients. From a survey among patients in the USA, the symptoms that were perceived as most debilitating were flushing, diarrhea, fatigue and the everyday risk of anaphylaxis.[52,91]

Next to these physical symptoms, previous studies have consistently shown an increased prevalence of psychiatric morbidity in patients with mastocytosis.[65,92] This might be caused by the effect of various mast cell mediators to the brain function.[92] However, it is still unclear whether psychological morbidity is a specific feature of mastocytosis, or whether it is a more general consequence of having a chronic disease with debilitating physical symptoms. In order to understand the type and extent of psychological symptoms in patients with mastocytosis, it appears useful to make comparisons with emotional and physical distress levels in patients with other diseases. In this study, we therefore aimed to investigate the presence of various psychological symptoms and quality of life in a representative cohort of patients with mastocytosis and compared these outcomes to already established reference

values for patients with chronic pain or cancer and healthy norm groups. Secondly, we analyzed the correlation between psychological symptoms, quality of life and mastocytosis-related characteristics.

Methods

Patients and procedures

A cross-sectional study was performed in 50 adult patients diagnosed with mastocytosis. This study was performed as an addition to a trial that investigated the prevalence and severity of acetylsalicylic acid among adult patients with mastocytosis.[93]M. A. W.</author><author>van der Vet, S. Q. A.</author><author>van Hagen, P. M.</author><author>van Wijk, R. G.</author><author>van Daele, P. L. A.</author></authors></contributors><auth-address>Department of internal medicine, section of Clinical Immunology, Erasmus University Medical Center, Rotterdam, The Netherlands.
Department of internal medicine, section of Allergy, Erasmus MC, Rotterdam, The Netherlands.</auth-address><titles><title>Low frequency of acetyl salicylic acid hypersensitivity in mastocytosis: the results of a double-blind, placebo-controlled challenge study</title><secondary-title>Allergy</secondary-title></titles><periodical><full-title>Allergy</full-title></periodical><edition>2018/03/24</edition><dates><year>2018</year><pub-dates><date>Mar 22</date></pub-dates></dates><isbn>1398-9995 (Electronic Therefore, the inclusion and exclusion criteria were based on that trial. Patients were recruited from the outpatient clinic of a tertiary university medical center, which is a national reference center for mastocytosis.. All patients under current treatment in the fall of 2016 received an invitation to participate by mail and were subsequently contacted by telephone. Adult patients with biopsy-proven cutaneous or systemic mastocytosis were eligible. All participants were asked to complete three questionnaires on psychological symptoms, health-related quality of life and general mastocytosis-related symptoms. The study was approved by the local medical ethics committee. All participants provided written informed consent.

Measures

Basic demographic characteristics and mastocytosis related characteristics such as subtype, presence of skin involvement and anaphylaxis, were recorded.

The participants completed the 90-item Symptom Checklist and the 36-item Short Form health survey. These questionnaires were used to screen for psychological symptoms and health-related quality of life from a patients' perspective. They are not intended to diagnose patients with specific psychiatric diseases.

The Symptom Checklist 90 (SCL-90) is a multidimensional psychopathology-indicator, that assesses to which extent patients were bothered by each item in the last week, including the day of the questionnaire.[94] The questionnaire consists of 90 items (symptoms) with scores ranging from 1 (not at all) to 5 (very). There are eight subscales: *anxiety, agoraphobia, depression, somatization, distrust and interpersonal sensitivity, hostility, sleeping problems* and *inadequacy of thinking and acting*. All items contribute to a total SCL-90 score of psychoneuroticism. Lower scores indicate a better state of recent mental health. Mean test scores of the study cohort were compared to scores of a healthy Dutch norm group and a norm group of patients with chronic pain, described by Arrindell and Ettema.[94] The internal consistency of the questionnaire is above 0.70, and most coefficient alpha's range from 0.80 to 1.00.[94]

The Short Form 36 (SF-36) is a multidimensional measuring instrument that captures the general state of health and functioning, physically as well as emotionally and socially.[95] Item scores are summoned and transformed into eight scale scores: *physical functioning, role limitations due to physical health, role limitations due to emotional problems, vitality, mental health, social role functioning, bodily pain and general health perception*. Overall, a physical health component and mental health component can be discerned. SF-36 outcomes are presented on a hundred-points scale. Higher scores indicate a better state of health. Mean test scores of the study cohort were compared to scores of a healthy Dutch norm group and a norm group of patients with cancer, described by Aaronson et al.[96] For all test scores the Dutch reference for women

was used. The mean coefficient alpha across scales and samples was 0.84.[96] Lastly, we asked patients to rate 18 mastocytosis-related symptoms in a self-developed questionnaire. These included pruritus, flushing, bone pain, myalgia, dyspepsia, diarrhea, collapse, dizziness, dyspnea, rhinitis, headache, insomnia, fever, night sweats, fatigue, brain fog, subjective cognitive problems, anddifficulties with memory. Symptoms were scored from 0 to 3 with 0 meaning 'never present', 1 meaning 'present ≤ three days a week', 2 meaning 'present ≥ three days a week', 3 meaning 'present every day'.

Analysis

IBM SPSS 25 was used for all analyses. Patient characteristics are given as mean with standard deviation (SD) for continuous variables and as the absolute number with percentage for dichotomous variables. The unequal variances 2-sided t-test was used to compare the mean test scores of the study cohort to norm scores of other populations.(11, 13) The 2-tailed Pearson correlation coefficient was calculated to investigate a correlation between test scores and continuous variables, and the independent samples t-test was used for dichotomous variables. For all tests α was set at 0.05. A few mastocytosis characteristics that were expected to be correlated to psychological disability, were compared to the main outcomes. These mastocytosis characteristics are tryptase levels, the presence of urticaria pigmentosa and symptoms of fatigue, diarrhea and anaphylaxis. The choice for these characteristics was based on previous literature [52,97] and our own experience from working with these patients. Test outcomes used for this analysis were: total SCL-90 score and SF-36 mental- and physical health components. By restricting the comparison analyses, a bias due to multiple testing was avoided. Procedures on missing items, provided by Arrindel and Ettema for the SCL-90, and by van der Zee and Sanderman for the SF-36, were followed.(11, 12) If, according to these guide lines, not enough items were answered, the patient was excluded from the analysis of the relevant questionnaire.

Table 1. Baseline characteristics study population (n=50).	
Age in years, mean (SD)	54.3 (12.8)
Male, n (%)	16 (32)
Educational level, n (%)	
• High school	2 (4)
• Secondary vocational education	27 (54)
• Higher professional education	16 (32)
• University education	5 (10)
Currently working‡, n (%)	28 (56)
• Not working due to health issues	9 (18)
• Retired	12 (24)
Subtype according to WHO criteria[33,98], n (%)	
• MIS	9 (18)[§]
• ISM	
with skin lesions	26 (52)
without skin lesions	9 (18)
• SSM	3 (6)
• SM-AHN	2 (4)
• ASM	1 (2)
Duration of disease in years, mean (SD)	19.2 (14.4)
Serum tryptase level at diagnosis in µg/l, mean (SD)	42.5 (47.8)
Daily mast cell mediator-related symptoms, n (%)	
• Pruritus	36 (72)
• Flushing	35 (70)
• Diarrhea	36 (72)
• Fatigue	43 (86)
• Anaphylaxis	22 (44)
• Osteoporosis†	8 (16)

[§]One patient underwent incomplete bone marrow investigation, 8 patients declined bone marrow punction.
†Osteoporosis was defined as a T-score $\leq$ 2.5 SD for bone densitometry of the lumbar spine and/or left femur.
‡One person is a housewife.

Results

Patient characteristics

Fifty patients were included. The mean age was 54.3 years (range 19-74 years), the majority of participants was female (68%) and most had indolent systemic mastocytosis (70%). Nine patients (18%) who were under the age of 65 years were not able to work due to health issues. The mean serum tryptase level was 42.5 µg/l, however with a wide range between 6.4 and 221.0 µg/l. The mean duration of disease was 19.2 years, also with a wide range of 74-2 years. Two patients used psychiatric medication, both a selective serotonin reuptake inhibitor. See Table 1 for all patient characteristics.

SCL-90

The study cohort scored significantly higher than the healthy norm group on the total SCL-90 score (p = 0.02), but lower than the norm group of chronic pain patients (p = 0.003). For the subscales of *depression, somatization, inadequacy of thinking and acting* and *sleeping problems*, the study population scored significantly worse than the healthy norm group (p = 0.04, 0.00002, 0.002, 0.001, respectively), but again lower than the patients with chronic pain (p = 0.007, 0.01, 0.0006, 0.006, respectively). There was no significant difference on

the subscales of *anxiety*, *agoraphobia* and *interpersonal sensitivity*. See figure 1 for a graphic summary. The exact mean subscale values with standard deviation are summarized in Online Supplementary eTable 1.

SF-36

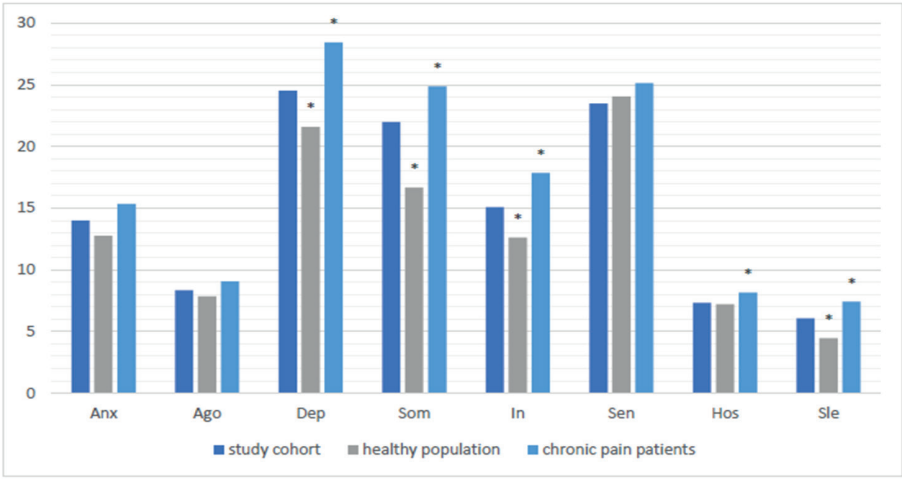


Figure 1. Mean SCL-90 subscale scores of the study cohort compared to norm scores of healthy persons and patients with chronic pain. *A higher score means more symptoms. The y-axis depicts the absolute mean score value. On the x-axis, the subscales of the Dutch SCL-90 subscales are shown. Anx = anxiety; Ago = agoraphobic symptoms; Dep = depression; Som = somatization; In = inadequacy of thinking and acting; Sen = interpersonal sensitivity; Hos = hostility; Sle = sleeping problems. *p <0.05 for comparison of the subscale value of the study population versus the other norm groups (t-test)*

The study cohort scored significantly worse than the healthy norm group on the subscales of *bodily pain, physical role functioning, social role functioning, vitality* and *general health perception* (p = 0.006, 0.001, 0.03, 0.0002, 0.00001, respectively). There was no difference in the subscales of *mental health, emotional role functioning and physical functioning* between the study cohort and the healthy norm group. The study cohort scored significantly worse than the norm group of cancer patients on *bodily pain* and similarly on *vitality* and *general health perception* (p = 0.03, 0.12, 0.64, respectively). However, cancer patients had significantly lower scores on *mental health*, *emotional role functioning* and *physical*

role functioning than our study cohort (p = 0.0004, 0.008, 0.04, respectively). See figure 2 for a graphic summary. The exact mean subscale values with standard deviation are summarized in Online Supplementary eTable 2.

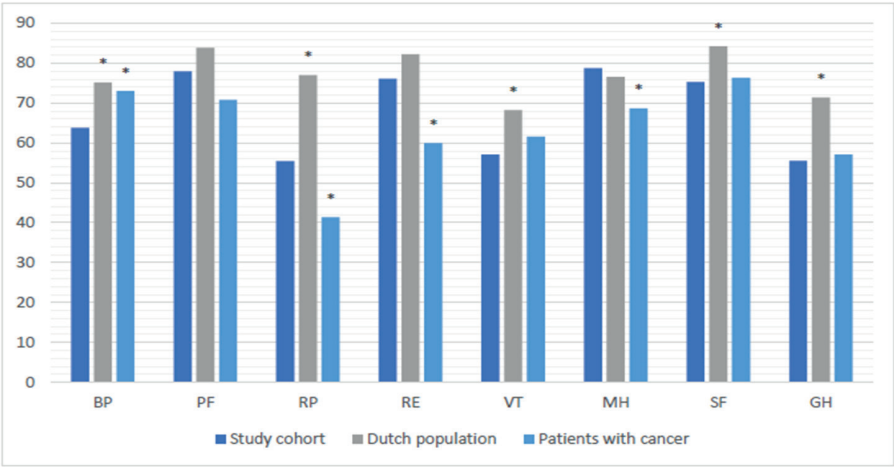


Figure 2. Mean SF-36 subscale values of the study cohort compared to SF-36 norm score of the Dutch population and cancer patients. *A lower score means lower quality of life on that area. The y-axis depicts the absolute score value. The subscales on the x-axis are shown as abbreviations of the SF-36 subscales. BP = bodily pain; PF = physical functioning; RP = physical role functioning; RE = emotional role functioning; VT = vitality; MH = mental health; SF = social role functioning; GH = general health perception. *p<0.05 for comparison of the subscale value of the study population versus the other norm groups (t-test)*

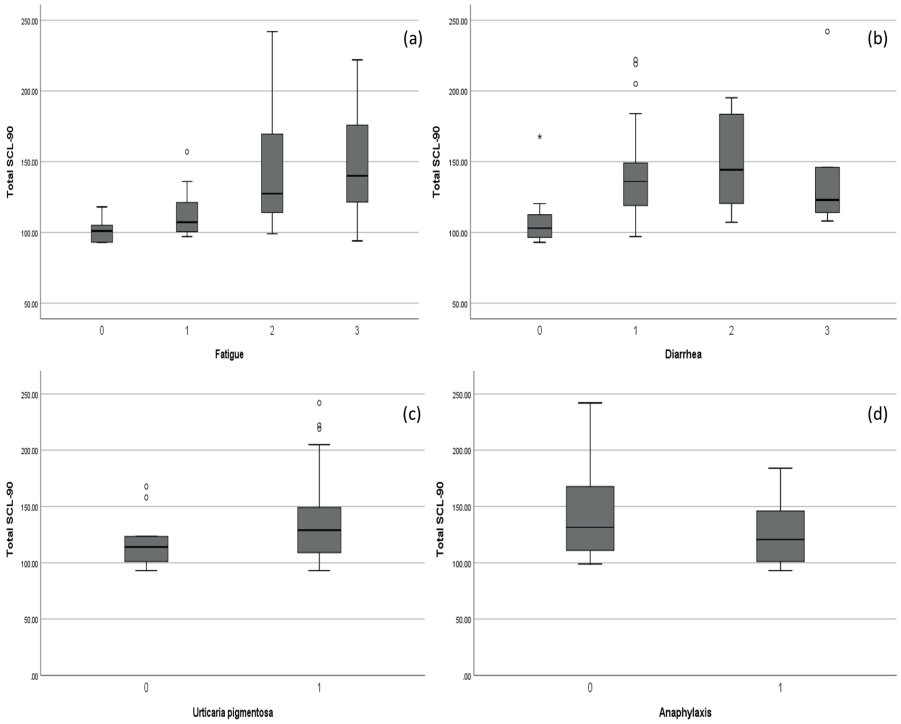
Correlation between psychological symptoms and mastocytosis-related characteristics

There was no significant correlation between the serum tryptase levels and the absolute scores of the total SCL-90 and the mental and physical component of the SF-36 (Pearson correlation coefficients 0.128, -0.021, -0.299 with p = 0.40, 0.90, 0.05, respectively). Graphical illustrations are given as Online Supplementary eFigure 1.

The presence of fatigue was significantly correlated with worse scores on the total SCL-90 as well as on the SF-36 mental component and physical component (Pearson correlation coefficients 0.461, -0.380, -0.328 with p = 0.001, 0.01, 0.03, respectively). Patients with diarrhea had significantly higher total

SCL-90 scores (Pearson correlation coefficient 0.342, p = 0.02). Diarrhea was not significantly correlated with worse absolute scores of the SF-36 mental and physical component (Pearson correlation coefficients -0.132, -0.088 with p = 0.40, 0.56, respectively). Furthermore, there was no significant correlation between urticaria pigmentosa and the absolute scores of the total SCL-90 and the mental and physical component of the SF-36 (p = 0.15, 0.52, 0.26, respectively). Finally, symptoms of anaphylaxis were not found to be significantly correlated to total SCL-90 scores and the mental and physical component of the SF-36 (p = 0.08, 0.36, 0.09, respectively). A graphical illustration of the correlations between these characteristics and the total SCL-90 is given in figure 3.

Figure 3. Graphical illustration of the association between the total SCL-90 score and a) fatigue (p 0.001), b) diarrhea (p 0.017), c) urticaria pigmentosa (p 0.15) and d) anaphylaxis (p 0.08).



Discussion

In this cross-sectional cohort study we investigated the presence and type of psychological symptoms of 50 adults with mastocytosis and their health-related quality of life. Secondly, we aimed to identify correlations with mastocytosis-related characteristics. This is the first study to compare psychological symptoms of patients with mastocytosis to historical norm groups of a healthy population and of patients with other illnesses that may greatly impact one's health-related quality of life, i.e. chronic pain or cancer. Patients with mastocytosis scored worse than the healthy norm group on the total SCL-90 scale as well as on its subscales of *depression*, *somatization*, *sleeping problems* and *inadequacy of acting and thinking*. The study cohort scored similar to a healthy norm group on the SF-36 subscales of *mental health* and *emotional role functioning* and similar to a norm group of patients with cancer on the SF-36 subscales of *general health perception* and *vitality* and even worse on the subscale of *bodily pain*.

Previous studies show that 30-64% of patients with ISM had symptoms of depression[66,99], and 38.6% had some degree of cognitive impairment.[65] In a questionnaire-based cohort study in the USA, 49.3% of patients reported some degree of depression, and 28.8% reported moderate to severe depression.[52] A similar prevalence of self-reported depression was found in a Dutch and another French cohort.[53,91] Since the designs of these studies differ from each other and from our study, it is difficult to directly compare the results. However, the rates of depression were considerable in every single study. Our study investigated mental and physical burden rather than the presence of specific psychiatric disorders in patients with mastocytosis. For example, the SCL-90 subscale of *depression* is not synonymous to the diagnosis of depression by a psychiatrist. We have used questionnaires that investigate psychological symptoms as well as the health-related quality of life of patients. . By comparing our cohort to scores of standard norm groups for the used questionnaires, we can confirm that patients with mastocytosis more often have psychological symptoms than healthy controls. However, our study cohort scored bet-

ter on most subscales of the SCL-90 than an established norm group with chronic pain. This may not be so surprising, as it is well known that chronic pain is strongly related to psychological functioning.[100] It must be noted that although the use of historical norm groups is in our opinion better than making no comparison at all, it is not certain that these groups are similar to the studied cohort regarding age, sex and other demographic characteristics. Moreover, the norm groups were obtained in the 90's and early 00's, and the general perception of health might have changed with time.

In general, our study population is a representative cohort of adults with mastocytosis, except for a relatively low number of male participants. Only two patients in our cohort were using antidepressants at the moment of obtaining the questionnaires, therefore this is probably not a significant factor in interpreting the results, but on the other hand emphasizing the underestimation of psychological burden in this patient category. Since we are an allergology-oriented clinic, relatively few patients with advanced subtypes of SM were included. Although the numbers were too small to perform statistical subgroup analyses, the patients with advanced SM did not seem to score worse on the different questionnaires than patients with MIS or ISM. This might be resultant of the fact that patients with advanced SM usually have less mast cell mediator-related symptoms.[101] Furthermore, only a few study patients had osteoporosis, which can cause chronic pain. The absence of chronic pain due to osteoporosis therefore might have influenced the SF-36 scores in a positive sense. A last possible limitation of our study is the use of self-report questionnaires. This could lead to both under- as overestimation, depending on the coping style of the included patients.

Strikingly, patients with mastocytosis have a similar perception of their general health to patients with cancer, while many physicians probably perceive mastocytosis as a benign and mild disease. The fact that 18% of the studied patients were not able to work due to their mastocytosis-related health issues stresses the debilitating nature of this disease in everyday life. Other studies

that investigated the quality of life of patients with mastocytosis reported that both fatigue as well as the unpredictability of symptoms such as anaphylaxis, diarrhea and flushing have a large influence on their quality of life.[97,102] Our results indeed showed positive correlations between fatigue, the total SCL-90 and the mental and physical component of the SF-36. Furthermore, the presence of diarrhea was significantly correlated to the total SCL-90 score. Correlations with serum tryptase levels, anaphylaxis and urticaria pigmentosa did not prove significant.

It appears logical that experiencing these debilitating symptoms on a daily basis has a negative influence on one's perception of overall health and can result in a depressed mood. Accordingly, other studies found a high prevalence of clinically significant fatigue among patients with mastocytosis, ranging from 43.4% to 62.4%.[52,63,103] Several phenomena could contribute to the increased perception of fatigue in patients with mastocytosis. Firstly, depressive symptoms often coincide with a feeling of fatigue.[104] Secondly, fatigue in the presence of chronic disease could also be explained in the context of the sickness behavior response. This is an automated response, triggered by innate immunity activation, to increase the survival of an animal by decreasing their activity.[105] However, as mastocytosis patients continuously have high levels of various mast cell mediators, this might exert direct effects on the brain. Recent research indeed showed an impaired tryptophan metabolism in patients with mastocytosis.[92] A small cohort study also showed a high prevalence of atypical white matter lesions in patients with mastocytosis (49%) and an increased perfusion of the putamen region compared with healthy controls.[99] The fact that treatment with the tyrosine kinase inhibitor masitinib has a positive effect on psychological symptoms suggests that depression may indeed be an endogenous manifestation of mastocytosis, rather than a sole secondary consequence of physical illness.[66]

However, this study was not designed to identify biological pathophysiological processes that cause psychological symptoms in mastocytosis. For future

studies, it would be interesting to combine all aspects mentioned above: neurobiological processes, the patient's own perspective, and the evaluation by an expert mental health professional.

Conclusion

Patients with mastocytosis score higher on psychological symptoms when compared to healthy controls, but lower than patients with chronic pain or cancer. More specifically, patients with mastocytosis significantly more often have depressive symptoms, sleeping problems, somatic complaints and inadequacy of acting and thinking, compared to healthy controls. Strikingly, patients with mastocytosis score similar to cancer patients on the SF-36 subscale of general health perception. These findings stress the importance of correct treatment of physical mastocytosis-related symptoms, as well as adequate psychological counselling for patients with mastocytosis.

Supplementary Material belonging to:
Psychological functioning and quality of life in patients with mastocytosis: a cross-sectional study.

Table 1. Comparison of mean SCL-90 subscale values for the study cohort, a healthy Dutch norm group and a norm group of patients with chronic pain.

	Study cohort (N=49†)	Healthy Dutch population (N=2368)	Patients with chronic pain (N=2458)
Anxiety ***Mean (SD)***	13.98 (4.86)	12.76 (4.41)	15.36 (6.29)
Agoraphobia ***Mean (SD)***	8.34 (2.94)	7.86 (2.34)	9.06 (3.95)
Depression ***Mean (SD)***	24.51 (9.66)	21.58 (7.56)*	28.43 (11.36)*
Somatization ***Mean (SD)***	21.97 (7.86)	16.68 (5.34)*	24.88 (7.93)*
Inadequacy of thinking and acting ***Mean (SD)***	15.10 (5.20)	12.63 (4.25)*	17.86 (6.40)*
Interpersonal sensitivity ***Mean (SD)***	23.48 (7.18)	24.05 (7.64)	25.15 (9.13)
Hostility ***Mean (SD)***	7.34 (2.09)	7.22 (2.10)	8.15 (3.11)*
Sleeping problems ***Mean (SD)***	6.07 (3.27)	4.46 (2.20)*	7.42 (3.67)*
Total SCL-90 ***Mean (SD)***	131.74 (36.95)	118,28 (32.38)*	148.57 (45.51)*

SCL-90: 90-item Symptom Checklist
† *One participant was excluded because of too many missing values*
**p <0.05 for comparison of the subscale value of the study population versus the other norm groups (t-test)*

Table 2. Comparison of mean SF-36 subscale values for the study cohort, a healthy Dutch norm group and a norm group of patients with cancer.

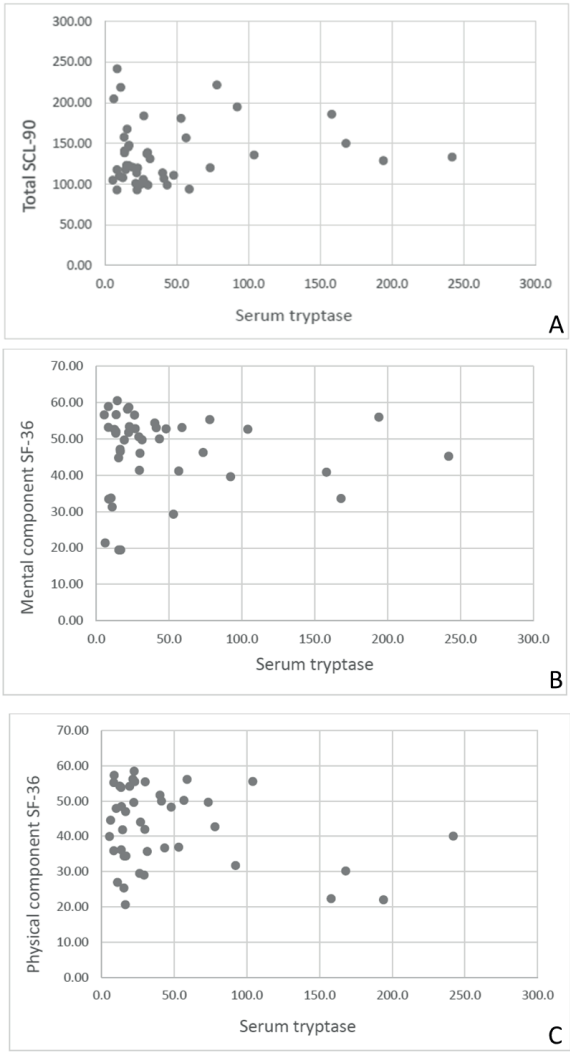
	Study cohort†	Dutch population	Patients with cancer
Bodily pain			
n	46	1729	481
Mean (SD)	63.78 (26.46)	75.1 (24.95)*	73.0 (21.93)*
Physical functioning			
n	46	1718	478
Mean (SD)	77.91 (23.22)	83.9 (20.72)	70.8 (21.86)
Physical role functioning			
n	46	1693	473
Mean (SD)	55.43 (42.79)	77.0 (37.03)*	41.4 (34.8)*
Emotional role functioning			
n	46	1686	474
Mean (SD)	76.09 (38.27)	82.2 (36.95)	60.0 (32.66)*
Vitality			
n	46	1715	479
Mean (SD)	57.03 (18.72)	68.2 (20.71)*	61.6 (19.7)
Mental health			
n	46	1714	479
Mean (SD)	78.74 (17.18)	76.5 (16.56)	68.7 (17.51)*
Social role functioning			
n	46	1729	481
Mean (SD)	75.27 (26.55)	84.2 (20.79)*	76.3 (21.93)
General health perception			
n	46	1705	479
Mean (SD)	55.54 (21.61)	71.4 (20.65)*	57.1 (19.70)

SF-36; SD: standard deviation.

† *Four participants were excluded because of too many missing values*

**p<0.05 for comparison of the subscale value of the study population versus the other norm groups (t-test)*

Figure S1. Graphical illustration of the association between serum tryptase and **A**) the total SCL-90 scale (p 0.396), **B**) the mental component of the SF-36 (p 0.894) and **C**) the physical component of the SF-36 (p 0.052).



Chapter 3

Dilemmas in diagnosticss

3.1 Systemic mastocytosis with normal serum tryptase: rule or exception?

M.A.W. Hermans, M.W.J. Schreurs, P.L.A. van Daele

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Systemic mastocytosis (SM) is a rare disease which is characterized by the accumulation of mast cells in ≥1 extra-cutaneous organ, most often the bone marrow.[33] In the majority of patients, the activating D816V mutation in KIT induces increased proliferation and survival in the neoplastic mast cells. The WHO has formulated criteria for the diagnosis of SM: Most criteria can usually only be obtained by bone marrow (BM) aspiration and biopsy.[33] Due to the heterogeneous clinical picture of SM, it can be challenging to determine when BM examination is indicated. Serum tryptase is often used as a screening tool for SM.[106] However, it is unclear at what value SM can be ruled out. The general international cut-off value is 11.4 ng/mL, whereas the WHO has established a level of ≥ 20 ng/mL as minor criterion for mastocytosis. Furthermore, an ECNM consensus paper included a tryptase level of 15 ng/mL in their diagnostic algorithm.[90]

We investigated whether a serum tryptase level ≤11.4 ng/mL sufficiently rules out SM in adults by retrospectively reviewing the files of all adults who visited the mastocytosis centre in the Erasmus MC from January 2009 to April 2019. Patients who fulfilled the WHO criteria for SM were included. All diagnostic procedures were performed according to the international consensus. Serum tryptase was analysed by FEIA technology on the Phadia250 system (Thermo Fisher Scientific, Uppsala, Sweden). Patients were divided into three categories according to tryptase levels: ≤ 11.4 ng/mL; 11.5 – 19.9 ng/mL; and ≥ 20 ng/mL.

Of the 238 patients who were evaluated, 198 patients had SM (figure 1). Thirty-six patients had probable SM but declined BM investigation. Four patients had

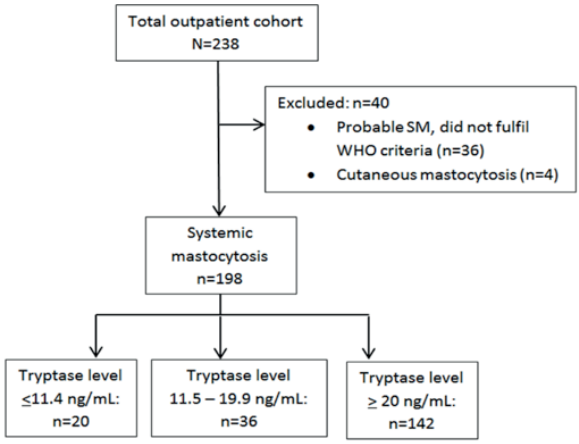

Figure 1: Flowchart of inclusion process.

CM, among whom the highest baseline tryptase level was 12.7 ng/mL. Three patients had onset of MPCM in infancy and one had a solitary skin lesion compatible with the now outdated diagnosis teleangiectasia macularis eruptiva perstans.

Table 1 summarises the characteristics of the 198 patients with SM. The median follow-up from the onset of symptoms was 16 years (IQR 15, range 1-58 years). The majority (46.4%) presented with MPCM. Anaphylaxis was the second most prevalent reason for further investigation (23.7%) and 7.2% presented with osteoporosis. Twenty patients (10.1%) had a tryptase level ≤11.4 ng/ml at diagnosis. Fifteen of them (75%) presented with MPCM, one patient presented with anaphylaxis, one with fatigue, one with hypereosinophilia, one with angioedema, and one with flushing. In the patients without MPCM, BM investigation was performed because D816V mutated cells were demonstrated in peripheral blood (n=2) or because of very high clinical suspicion of SM (n=3). All patients with advanced forms of SM had a tryptase level >11.4 ng/mL. There was a trend towards more skin involvement and less anaphylaxis in the group with a serum tryptase ≤ 11.4 ng/mL, but statistical significance was not reached. In eight patients (33.3%) with a tryptase level ≤11.4 ng/mL at diagnosis, a tryptase level >11.4 ng/mL was measured some time during follow-up. Of the 142 patients with a tryptase level ≥20 ng/mL at diagnosis, seven had a tryptase level ≤11.4 ng/mL somewhere during follow-up without mast cell reductive pharmacotherapy. Thus, tryptase levels can significantly fluctuate over time.

Here, we show that 10.1% of patients with SM have a baseline tryptase level ≤11.4 ng/mL. There were no significant differences in clinical characteristics between the three categories of serum basal tryptase levels, but this could be due to the small group numbers. Few studies have yet investigated the diagnostic accuracy of serum tryptase measurement. Two papers that described the prevalence of SM among adults with MPCM found that 39% and 24.2% of patients with SM had a serum tryptase level <20 ng/mL.[35,37] This is very comparable to our results. In another cohort of ISM patients without skin involvement, the lowest tryptase level found was 16.7 ng/mL.[15] This might

represent a diagnostic bias, if patients with a tryptase level ≤ 11.4 ng/mL were excluded of further screening. In recent years, peripheral blood D816V PCR was developed as an additional means to sensitively screen for mastocytosis and it is rapidly finding its way into daily practice.[43] Studies investigating the sensitivity of this PCR confirm that a normal tryptase level does not rule out SM in individuals with adult-onset MPCM or Hymenoptera venom-related anaphylaxis.[107,108]

In conclusion, 28.3% of adults with SM had a serum tryptase level below 20 ng/mL at diagnosis. It can be questioned whether the current WHO minor criterion of a tryptase level ≥20 ng/mL should be adjusted. We advocate complete BM examination in every patient with a tryptase level >11.4 ng/mL. Further investigation should also be considered in patients with a tryptase level ≤11.4 ng/mL with clinical symptoms that are suggestive of mastocytosis, especially adult-onset MPCM or Hymenoptera venom-related anaphylaxis.

Table 1. Patient characteristics according to serum tryptase levels at diagnosis.

	Tryptase ≤ 11.4 ng/mL N=20	Tryptase 11.5 – 19.9 ng/mL N=36	Tryptase ≥ 20 ng/mL N=142
Age at diagnosis in years, median (IQR)	44 (17)	50 (19)	51 (15)
Male, n (%)	6 (30)	12 (33.3)	64 (45)
Years to diagnosis, median (IQR)	10 (9)	4.5 (10.5)	4 (11)
BMI, median (IQR)	23.8 (5.5)	27.2 (6.3)	28.4 (7.0)
Diagnostic results positive, n (%)*			
- **BM biopsy**	16/17 (94.1)	19/21 (90.5)	102/111 (91.9)
- **BM aspirate**	16/19 (84.2)	30/32 (93.8)	126/134 (94.0)
- **Flowcytometry†**	17/18 (94.4)	24/28 (85.7)	101/104 (97.1)
- **D816V KIT detectable**	14/17 (82.4)	22/27 (81.5)	79/92 (85.9)
Mastocytosis subtype, n(%)			
- **ISM**	20 (100)	32 (88.8)	118 (83.1)
- **SSM**	0	0	5 (3.5)
- **SM-AHN**	0	3 (8.3)	9 (6.4)
- **ASM**	0	1 (2.8)	7 (4.9)
- **MCL**	0	0	2 (1.4)
Skin involvement, n (%)	16 (80)	24 (66.7)	96 (68.1)
Anaphylaxis, n (%)	4 (20)	17 (47.2)	51 (35.9)
Flushing, n (%)	13 (65)	18 (50)	63 (44.4)
Dyspepsia , n (%)	8 (40)	11 (30.6)	57 (40.1)
Diarrhoea, n (%)	9 (45)	13 (36.1)	51 (36)
Fatigue, n (%)	11 (55)	18 (50)	75 (45.1)
Atopy, n (%)	3 (15)	2 (5.6)	10 (7)
Hepatosplenomegaly, n (%)¶	2 (10)	2 (5.6)	19 (13.4)
Osteoporosis, n (%)	3/20 (15.0)	5 (13.9)	36/134 (26.9)
Bone marrow density femur, median (IQR)	-0.55 (1.0)	-0.50 (1.8)	-0.60 (1.30)

ASM = aggressive systemic mastocytosis, BM=bone marrow, CM=cutaneous mastocytosis, ISM systemic mastocytosis, IQR=interquartile range, MCL=mast cell leukemia, SM-AHN=systemic m with associated haematological neoplasm, SSM=smouldering systemic mastocytosis.

*Bone marrow investigation was not always completely performed, as patients were often ref other clinics after the diagnosis of mastocytosis was made.

†Flowcytometry is considered positive when CD2 and/or CD25 expression was proven on mas

¶As determined by ultrasonography at diagnosis.

3.2 The basophil activation test is not a useful screening tool for Hymenoptera venom-related anaphylaxis in patients with systemic mastocytosis.

M.J.A. Rietveld, M.W.J. Schreurs, R. Gerth van Wijk, P.L.A. van Daele, M.A.W. Hermans

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Abstract

Background: Systemic mastocytosis (SM) patients are at high risk of anaphylaxis, with Hymenoptera as main culprit. A screening instrument to identify which patients are sensitized to Hymenoptera, before they experience anaphylaxis, would therefore be of great value. Basophil Activation Test (BAT) is proposed as a possible tool for diagnosing Hymenoptera venom-related allergy (HVA), especially in patients in whom conventional allergy tests give contradictory results.

Methods: We included outpatients with SM, according to the WHO criteria, in a period of September 2011 to January 2012. Next to obtaining various clinical data including intradermal test results, sIgE measurement and BAT were performed.

Results: We included 29 patients, of whom 9 had a history of HVA and 4 had experienced anaphylaxis due to other triggers. 16 patients had no history of anaphylaxis. sIgE was detected in 6 patients with HVA and in 2 patients with anaphylaxis due to other triggers. BAT was positive in only 1 patient, in whom the skin test and sIgE were also positive. Compared with patients with skin lesions, those without skin lesions had significantly more anaphylaxis and sIgE to Hymenoptera. During three year follow-up, no-one experienced new anaphylactic episodes.

Conclusion: BAT is not a reliable tool for randomly screening SM patients for HVA.

Introduction

Systemic mastocytosis (SM) is a rare disease which is characterized by proliferation of aberrant mast cells in which at least one extracutaneous organ is affected. [109] Since mast cells are the culprit cells of type I hypersensitivity reactions, SM patients are at a high risk of anaphylaxis with a cumulative incidence ranging between 20 and 49%. [47,48,110] Another study showed that 12% of patients of a cohort had life-threatening anaphylaxis, sometimes with serious cerebral hypoxic damage.[51] Patients with skin lesions have a lower lifetime risk of anaphylaxis than patients without skin lesions.[77] Many triggers can cause anaphylactic reactions, but in SM patients most are Hymenoptera-venom related .[51] This can obviously lead to life threatening situations. Therefore, it would be of great value to determine those patients that are sensitized to Hymenoptera venom before they experience anaphylaxis, and maybe even preemptively treat them with immunotherapy. Conventional tests including intradermal tests and measurement of specific IgE (sIgE) in serum are feasible to confirm sensitization after a patient has experienced an anaphylactic reaction, but are not currently deemed useful for screening purposes. In particular, the presence of sIgE does not always predict Hymenoptera-related anaphylaxis (HVA). The basophil activation test (BAT) has been proposed as a useful adjunct in the diagnosis of allergic disease, especially in patients with negative or contradictory conventional tests. [111-114] In BAT, basophils are used as an *in vitro* model for mast cells, despite their slightly different characteristics regarding appearance and function. Both however contain granules of preformed molecules that can cause anaphylactic reaction after degranulation. Degranulation occurs after a wide range of stimuli, for instance activation by IgE, complement mediators or bacterial-derived peptides.[81] Using BAT, both IgE-mediated as well as IgE-independent type-1-hypersensitivity can be measured *in vitro*. [115-117]. In previous studies, BAT has varying diagnostic characteristics when compared with intradermal tests and sIgE measurement in populations with, as well as without, mastocytosis. [118,119]

Materials and methods

The objective of this study was to determine whether BAT is also applicable for screening SM patients for Hymenoptera venom sensitization, and thereby, their risk for anaphylaxis due to a wasp sting. We used sIgE as a control measurement to determine who was sensitized to Hymenoptera venom.

Subjects

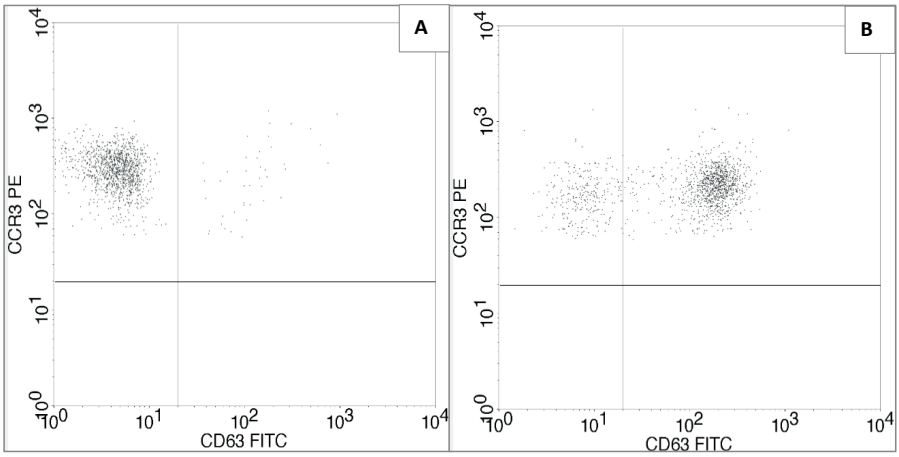
In the period of September 2011 to January 2012, we prospectively included patients who visited the outpatient clinic for routine visits and who fulfilled the WHO criteria for systemic mastocytosis.[15] We collected both demographic and disease related data including personal characteristics, subtype of systemic mastocytosis, and a detailed history regarding anaphylaxis. All patients gave informed consent. We retrospectively checked all patient files until September 2015 (3 years) for new episodes of anaphylaxis.

Basophil Activation Test (BAT)

Venom-activated basophils were identified by flow cytometry using the Flow2 CAST system (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland), according to the manufacturer's instructions. Briefly, 50 µL EDTA anticoagulated whole blood of the patient was incubated for 25 minutes at 37°C with 50 µL venom extract diluted to 100 ng/mL, in the presence of 100 µL stimulation buffer containing calcium and IL-3. We used Hymenoptera venom included in the Flow2 CAST kit. Subsequently, 20 µL fluorochrome-labeled monoclonal anti-CD63 antibodies and anti-CCR3 antibodies (staining reagent) was added (Bühlmann Laboratories, Schönenbuch, Switzerland). After washing the samples, flow cytometry was performed using a FACSCalibur (BD BioScience, New Jersey, USA) to detect degranulated CCR3 positive basophils based on the amount of CD63 expression. We used stimulation buffer as negative control and the positive control consisted of anti-FcεRI antibodies.(all Bühlmann Laboratories, Schönenbuch, Switzerland). A <15% increase in CD63 positive basophils, compared with the negative control was considered negative. A >15% increase in CD63 positive basophils was considered a positive result.

Results were only considered suitable for analysis when of the negative and the the positive controls gave the expected results. Figure 1a shows a flow cytometry plot of patient-derived leukocytes indicating CCR3 positive basophils (selected), either <15% degranulated (1b) or >15% degranulated (1c), based on CD63 expression.

Figure 1. Flow cytometry plots of a negative BAT (**A**) and a positive BAT (**B**), according to the surface CD63 expression on basophils.



Specific IgE measurement

Specific IgE (sIgE) antibodies against bee venom (i1) and wasp venom (i3) were determined using the Phadia 250 system (Thermo Fisher Scientific/ Phadia B.V. , Freiburg, Germany). Values of sIgE below 0.35 kU/L were considered negative. All analyses were performed according to manufacturer's instructions.

Statistical analysis

SPSS 21 (IBM SPSS Inc, IL, USA) was used for all statistical analyses. Values are reported as median with range, or median with standard deviation. We used chi-square and the t-test to compare different subgroups. However, because of the small population, we only performed statistical analysis on relevant characteristics.

Results

We included 29 patients (Table 1). The median age was 48 years (range 25-72) and 17 patients were female (59%). None of the patients used systemic corticosteroids at the time of our study. Patients with a history of anaphylaxis had a lower median serum tryptase level (p 0.438) than patients without anaphylaxis, not reaching statistical significance. Of the 13 patients who had experienced at least one anaphylactic reaction, 9 patients reported a wasp sting as the trigger. 3 patients had food-related anaphylaxis, after ingestion of prawn, hazelnut or peanut respectively. In 1 patient, the trigger for anaphylaxis was unidentified.

Appropriate skin tests were performed in all patients with reported anaphylaxis after a wasp sting. In 2 of these 9 patients, skin tests were negative. These patients also had undetectable sIgE for either wasp or bee venom.

Of all 9 patients who reported Hymenoptera-related anaphylaxis, 5 had detectable sIgE levels for Hymenoptera venom. One of these patients had detectable sIgE for bee venom, without a clinical history of bee-venom related anaphylaxis. Skin testing in this patient was positive for wasp, but negative for bee venom, thus the sIgE for bee venom probably represents irrelevant cross-reactivity with sIgE for wasp venom. Interestingly, 1 patient who reported anaphylaxis due to medication or prawn, also had detectable sIgE for Hymenoptera venom.

All BATs were considered suitable for analysis. Of all 29 patients, the BAT was positive in only one patient. This patient had ISMs-, and had experienced wasp-related anaphylaxis 2 years before inclusion in our study. Of note, she also had the highest level of sIgE to Hymenoptera venom of our population (4.61 kU/l) and positive intradermal test for wasp venom. sIgE for bee venom was not detectable in this patient. In the 3 year follow-up period, 1 patient was lost to follow-up. From the other 28 patients, no-one reported another wasp sting, nor did they experience new anaphylaxis.

Lastly, data were viewed in a different perspective for additional information. Irrespective of their clinical history of anaphylaxis, patients with ISM were

further divided in patients with or without skin lesions (ISMs+ vs. ISMs-, respectively). Anaphylaxis was significantly more frequent in ISMs- vs. ISMs+ patients (6/7 vs. 7/20, p 0.021). In accordance with this, sIgE to Hymenoptera venom also was significantly more often detectable in ISMs- vs. ISMs+ patients (5/7 vs. 2/20, p 0.001).

Table 1. Patient characteristics.

	No history of anaphylaxis (n=16)	**History of anaphylaxis not Hymenoptera-related (n=4)**	**Hymenoptera-related anaphylaxis (n=9)**
Age at diagnosis in years*	44 (25 – 56)	50 (38 – 63)	52.5 (36 – 72)
Female gender (n)	9/16	4/4	4/9
Time anaphylaxis to BAT in years*	N/A	2 (0.5 – 6)	3 (2 – 10)
Wasp sting after diagnosis	0/14	0/4	0/8
Wasp immunotherapy before BAT	N/A	0/4	1/9
Subtype systemic mastocytosis (n) †			
ISM	14/16	4/4	9/9
ISMs+	12/14	2/2	5/9
ISMs-	2/14	2/2	4/9
ASM	2/2	0/0	0/0
WHO criteria [13]‡			
Skin lesions (n)	14/16	2/4	5/9
Bone marrow histology positive (n)	5/6¶	1/1	3/3
Bone marrow morphology positive (n)	10/10	2/2	8/8
Tryptase at diagnosis in ng/L*	38,7 (9,8 – 324,0)	31 (22.6 – 44.1)	23.7 (15.9 – 160)
D816V mutation detected (n)	6/8	2/3	6/6
CD2 or CD25 detected (n)	8/8	2/2	6/6
Conventional allergic tests			
Intradermal tests for wasp positive (n)	N/A	0/4	7/9
Specific IgE to Hymenoptera venom (n)	0/16	1/4	6/9
Specific IgE levels in kU/l*	-	0.29	0.60 (0.22-4.61)
Specific IgE to bee venom (n)	0/16	0/16	1/9

* Values are given as median (range).

† ISM: indolent systemic mastocytosis. ISMs+: ISM with skin lesions. ISMs-: ISM without skin lesions. ASM: aggressive systemic mastocytosis. N/A: not applicable

‡ Some data are missing because initial workup for SM was performed in other medical centres.

¶ 1 patient had negative bone marrow biopsy for SM, but fulfilled all three minor WHO criteria for SM.

Discussion

We studied the feasibility of BAT as a screening tool for Hymenoptera sensitization in patients with SM. Of all patients, only 1 had a positive BAT and this patient also tested positive for the conventional allergy tests (sIgE and skin test). However, 8 other patients with a clinical history of HVA, of whom 5 also had demonstrable sIgE to Hymenoptera and 6 had positive intradermal tests, tested negative with BAT. These tests thus did not correlate well in our population, nor did BAT add useful information to the standard combination of clinical history, sIgE and skin tests. In addition to new data about the BAT, our studie provides additional evidence on the difference between ISMs+ and ISMs- patients. It is becoming increasingly clear in recent research that these two subtypes of ISM have entirely different clinical phenotypes. [6 + unpublished data] Our results confirm that anaphylaxis is more common in ISMs- patients vs. ISMs+.

The role and usefulness of BAT remains a topic of discussion in the current literature with earlier studies reporting conflicting evidence. Bidad et al. studied the role of BAT in diagnosing and monitoring of HVA in SM patients. [119] They found a sensitivity of 87% and a specificity of 100% in their population. However, all patients with HVA also had a positive intradermal test, rendering the addition of BAT redundant. Moreover, sIgE was significantly higher in this group compared with our population. We would therefore postulate that BAT does not add useful information to the conventional diagnostic tests for HVA. This hypothesis is supported by the results of Bonadonna et al. [120]. They investigated the role of BAT in SM patients with or without a reported reaction to Hymenoptera stings but all without demonstrable sIgE. In this population, BAT results were all negative and performing a BAT on top of sIgE and intradermal tests did not contribute to the diagnosis of HVA. Furthermore, Gonzalez et al. investigated BAT in patients with HVA and SM compared with patients with HVA but no SM. [121] Specific IgE was detected in 15 out of 22 SM patients, of whom 9 had a positive BAT result. Of the 7 SM patients without sIgE, 3 had a positive BAT. By using extracts of different wasp and bee spe-

cies, they even found the culprit insect in two of these sIgE-negative patients. Another study by Eberlein-König et al. also concludes that they were able to identify the culprit insect by BAT in a few complicated cases in which intradermal tests and sIgE measurement could not lead to a diagnosis. [112]

In addition to the different populations studied, an explanation for the strikingly different outcomes in all these studies can be found in technical differences. The BAT has not been standardized completely yet, and different laboratories use different techniques. For instance, some laboratories use purified cells in their analyses which can increase the sensitivity of BAT. [122] Cut off values and incubation substances also vary. Moreover, when working with basophils, one has to realize that basophils differ from (neoplastic) mast cells in certain ways, mainly non-IgE-mediated activation.[123] The main limitation of this specific study is the small number of patients investigated and the heterogeneity within our population. However, we chose this method on purpose to resemble daily practice. Moreover, the main aim was to investigate the role of BAT as a screening method, rather than to confirm the presence of an allergy that had already been confirmed by either clinical anaphylaxis or conventional tests. Therefore, we also included patients without a history of anaphylaxis which leads to a lower a priori incidence of HVA and, thereby, a lower sensitivity of BAT in this group.
Lastly, two patients with HVA had received immunotherapy several years before this study, which can obviously influence the results of BAT, since this is expected to become negative after immunotherapy. One of these patients, however, experienced an episode of wasp-related anaphylaxis after immunotherapy and therefore the effect of immunotherapy apparently was negligible.

Conclusions

Considering our results in relation to previous studies, we prudently conclude that BAT is not a useful test to screen random SM patients for their risk of HVA. Furthermore, by combining the patient's clinical history, intradermal testing and sIgE measurement, a diagnosis of HVA can be confirmed or

declined in most patients. BAT may be of additional use in very complicated patients with conflicting results of conventional tests, but we could not confirm this in our study. More systematic research in a carefully selected population might provide more insight in the role of BAT in this niche.

3.3 Abdominal ultrasonography has limited value in the care for patients with indolent systemic mastocytosis.

C.L. de Mol, M.A.W. Hermans, R. Gerth van Wijk, P.M. van Hagen, P.L.A. van Daele

Hematology 2017 Oct;22(9):544-547

Abstract

Objectives: Systemic mastocytosis (SM) is a myeloproliferative disease characterized by the accumulation of aberrant mast cells. Since advanced subtypes of SM can lead to organ dysfunction and shortened survival, timely recognition of progressive disease is important for the adequate treatment of SM patients.

Methods: Here, we report the results of our cohort study on the value of routine abdominal ultrasonography for the detection of progression of indolent systemic mastocytosis (ISM).

Results: We included 95 patients with SM, of whom 9 developed new hepatosplenomegaly during follow-up. In this group, the median serum tryptase level increased by 11.60 μg/L, compared with a decrease of -0.20 μg/L in the 79 patients with unchanged ultrasonography results (p=0.016). A change in liver and/or spleen size never led to a change in clinical classification, nor management.

Discussion: Based on the finding that a change in ultrasonography findings did not correlate to disease progression in general, it appears that isolated hepatosplenomegaly does not have prognostic implications in patients with ISM.

Conclusions: Routine abdominal ultrasonography is redundant in the follow-up of patients with SM. A combination of physical examination with serum tryptase levels can be used to screen for hepatosplenomegaly.

Introduction

Mastocytosis is a rare myeloproliferative disease in which there is uncontrolled proliferation of aberrant mast cells.[1] The World Health Organisation (WHO) distinguishes cutaneous from systemic mastocytosis. [76] Systemic mastocytosis (SM) is further divided into different subtypes, each having their own criteria, clinical manifestations, and prognosis.[40,124] Indolent systemic mastocytosis (ISM) is the most common subtype which has a favourable prognosis, with patients mainly suffering from mast cell mediator-related symptoms. More recently, it was recognized that the clinical symptoms of ISM patients with skin lesions are distinct from the ISM patients without skin lesions (ISMs+ vs. ISMs-, respectively).[103] In contrast to ISM, advanced subtypes are characterized by organ infiltration by mast cells (smouldering SM and aggressive SM), or a second haematological (non-mast cell) neoplasm (SM-AHN). To diagnose smouldering SM, 2 or more B-findings have to be present: hepato- or splenomegaly, lymphadenopathy, >30% mast cell infiltration of bone marrow, a serum tryptase level >200 μg/L, or signs of dysplasia without reaching criteria for myelodysplastic syndrome. For aggressive SM, C-findings were formulated: cytopaenia, signs of liver cirrhosis, malabsorption, or osteolytical bone lesions. The difference between smouldering and aggressive SM is the respective absence or presence of organ dysfunction. These advanced subtypes can shorten survival and often warrant cytoreductive treatment. [75,124] Hence, an important goal of outpatient follow-up of SM patients is to screen for progression of the disease or development of a second haematological disease. (3) There is some evidence that radiological examination of the abdomen can aid in determining the extent of systemic involvement of SM. [125] For these reasons, a consensus group of Dutch physicians with expertise in mastocytosis previously decided to routinely perform abdominal ultrasonography in the follow-up of SM patients. In the Erasmus University Medical Centre, it was common practice to perform abdominal ultrasonography every 2-3 years to screen for hepatosplenomegaly. However, new data show that progression of indolent to advanced SM is rare, and it is unclear what would be the best method to screen patients with ISM for progression. Moreover, in this era where health care costs are an important societal

issue, it is important to cut down unnecessary tests. The objective of this study was therefore to determine the value of routine abdominal ultrasonography as a tool to screen for progression of indolent to more aggressive forms of SM.

Methods

Patient selection and data collection

We selected all adult patients who visited the Erasmus University Medical Centre from January 2009 to February 2016 and who fulfilled the WHO criteria for ISM. [76] Only patients who underwent at least 2 abdominal ultrasonography's, with a minimal interval of 2 years, were included. Patients who had SM-AHN or ASM at baseline were excluded. All patients visited the outpatient clinic at least once a year. Bone densitometry was performed every 3 years to screen for osteoporosis. We retrospectively collected the findings on abdominal ultrasonography, as well as data on patient baseline characteristics, SM subtype and symptoms, tryptase levels and bone densitometry results from the electronic patient charts.

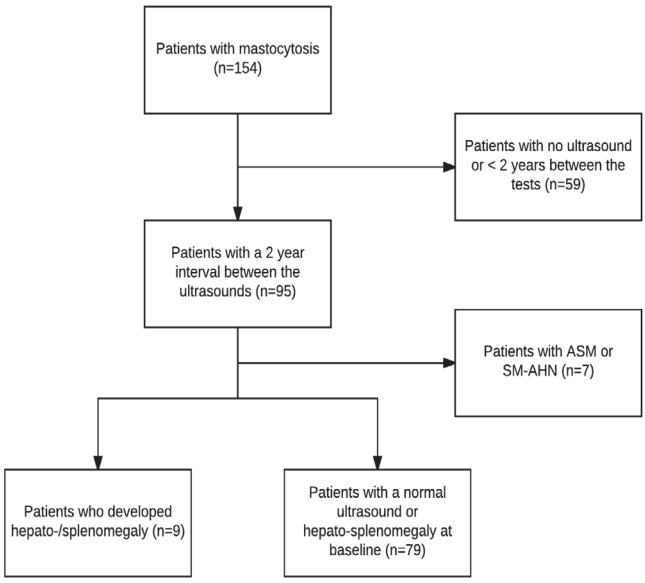


Figure 1. Flow chart of the inclusion process.

Definitions

Hepatosplenomegaly was defined as either a craniocaudal liver size of >15 cm., and/or a spleen size of >11 cm. A change in ultrasonography findings was defined as a change from a normal size to hepatosplenomegaly on subsequent examinations, or vice versa. Cytopenia was defined as either anaemia (Hb <10 g/dL), leukopenia (<400 × 10^6) and/or thrombocytopenia (<1,000 × 10^6). Skin involvement was described as all forms of maculopapular cutaneous mastocytosis (MPCM).[36]

Statistical analysis

We constructed 2 groups, according to their course of ultrasonography results. The primary endpoint was progression from indolent to smouldering, aggressive or SM-AHN, or progression from smouldering to aggressive or SM-AHN. We used IBM SPSS statistics 21 for all analyses. The Mann-Whitney U test was used for the comparison of the tryptase values and unpaired t-tests were used for continuous variables. The chi-square test was used for dichotomous variables.

Results

Study population

Of a total of 154 patients, 95 underwent multiple abdominal ultrasonography's with an interval of ≥2 years. Seven patients were excluded because they fulfilled the WHO criteria for ASM or SM-AHN at baseline. The population we analysed thus consisted of 88 patients, of whom 81 had ISM and 7 patients had smouldering SM. Ten of these 88 patients had a change in liver and/or spleen size over time, 9 of whom newly developed hepatosplenomegaly (10.2% of the total population). The liver and spleen size normalised in 1 patient. The median follow-up time was 11.20 years.

Group characteristics

We divided the population into two groups, entitled 'unchanged hepatosplenomegaly status' and 'new hepatosplenomegaly'. Baseline characteristics and

follow-up data are summarised in table 1. The patient with normalisation of liver/spleen size was included in the 'unchanged hepatosplenomegaly status' group. The age of the patients did not differ significantly between the two groups. Two patients in the 'unchanged hepatosplenomegaly status' group had cytopenia at baseline. No-one had other B- or C-findings. The development of hepatosplenomegaly during follow-up was not associated with a decrease in bone density.

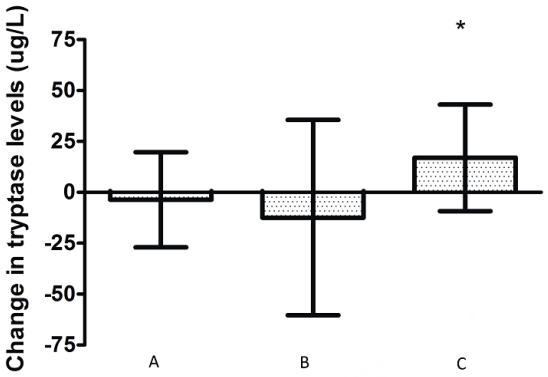


Figure 2. Change in serum tryptase levels during follow-up correlated with the development of new hepatosplenomegaly.
A) No hepatosplenomegaly; **B**) Hepatosplenomegaly at baseline; **C**) New hepatosplenomegaly during follow-up.
*p<0.05 compared with group A.

Follow-up findings

The median change in serum tryptase level during follow-up was -0.20 μg/L (SD 28.3) for the 'unchanged hepatosplenomegaly status' group, versus an increase of 11.60 μg/L (SD 26.2) in the 'new hepatosplenomegaly' group (figure 2). When comparing the change in tryptase levels over time, a significant difference was found between the patients who newly developed hepatosplenomegaly compared with the patients with unchanged ultrasonography findings (p=0.016). In the patients who newly developed hepatosplenomegaly, there was no statistically significant change in liver enzyme levels (mean

increase in ASAT levels of 4 U/L (SD 11.86 U/L), mean increase of ALAT levels of 7.5 U/L (SD 22.05 U/L)).

Of the patients with 'unchanged hepatosplenomegaly status', 12 (15.2%) had hepatosplenomegaly at baseline which did not change during follow-up. These patients did not develop clinical signs of liver cirrhosis during follow-up, nor did they show other signs of progression of mastocytosis. Accordingly, serum tryptase levels remained relatively stable with a median decrease of – 1.15 μg/L (SD 48.0 μg/L) in this subgroup.

One patient in the unchanged group went from ISM s+ to smouldering SM over time, based on a serum tryptase level of >200 μg/L and >30% bone marrow infiltration by mast cells. This patient had no hepatosplenomegaly. A change in liver and/or spleen size alone never led to a change in SM subtype, nor in a change in the medical management. One patient showed normalisation of his liver- and spleen size. This patient was treated with imatinib because of extensive skin lesions. In this patient the KIT D816V mutation was detected and his tryptase value increased by 6.0 μg/L during follow-up.

Table 1: Patient characteristics, grouped according to ultrasonography findings.

	Unchanged status of hepato-splenomegaly (n=79)	New hepato-splenomegaly (n=9)	p-value
Age in years (median, SD)	58.00 (12.89)	54.00 (9.94)	NS
Male sex (n, %)	35 (44.3%)	3 (33.3%)	NS
Follow-up time in years (median, SD)	10.00 (6.76)	12.00 (3.24)	NS
Subtype of SM:			N/A
- ISM s-	17	2	
- ISM s+	56	6	
- SSM	6	1	
Absolute change in serum tryptase levels in µg/L (median, SD)	-0.20 (28.3)	11.60 (26.2)	0.016
Change in subtype (n)	1	0	N/A
Change in treatment (n)	0	0	N/A
Cytopenia at baseline (n,%)	2 (2.5%)	0 (0%)	NS
Decrease in bone density during follow-up (n,%)	11 (13.9%)	0 (0%)	NS

Discussion

This study shows that routine abdominal ultrasonography has limited value in the follow-up of patients with indolent systemic mastocytosis: 10.2% developed new hepatosplenomegaly, but no-one showed progression to a more advanced type of SM. The development of new hepatosplenomegaly had no clinical consequences in any of them. Until this study, abdominal ultrasonography was part of the routine follow-up in the Erasmus University Medical Centre to screen for progression of ISM. However, it has become clear that progression of ISM is very rare. [40,86] Our results confirm this, as only one patient (1.1%) progressed from indolent to smouldering SM in a median follow-up time of 11.20 years. Moreover, this patient did not develop hepatosplenomegaly. Furthermore, patients who had hepatosplenomegaly at baseline

did not develop clinical signs of liver cirrhosis, or progression of mastocytosis in general. Based on these findings, we hypothesise that the finding of isolated hepatosplenomegaly, without other B- or C-findings, does not have important prognostic implications. Therefore, looking for hepatosplenomegaly is probably not appropriate when screening for progression of disease in ISM. Moreover, badly indicated radiologic investigations lead to higher health care costs, and a risk of unwanted incidental findings. [126] Interestingly, the median serum tryptase level increased significantly in patients who newly developed hepatosplenomegaly, whereas they remained stable in patients with unchanged ultrasonography findings. Besides the serum tryptase level, there were no other signs of progression of disease in all patients but one who progressed from indolent to smouldering SM. More specifically, patients did not develop other B- or C-findings. In another study, serum tryptase levels did correlate to clinical progression in general, and patients with rising serum tryptase levels more often developed hepatosplenomegaly.[127] It would be interesting to know whether the hepatosplenomegaly truly is mast cell related, however, this would require biopsies of both organs which is a risky procedure and not feasible in this context. Furthermore, MR elastography or fibroscan could be of additional value to estimate a risk of liver cirrhosis in the future. Unfortunately, we have not performed these investigations routinely and cannot provide data on this yet.[128]

To our knowledge, our study is the first to focus on the value of routine abdominal ultrasonography in the screening for progression of disease in ISM patients. However, our results should not be extrapolated to patients with advanced subtypes of SM. Follow-up abdominal ultrasonography can be indicated in advanced SM for other reasons. One limitation of this study is the retrospective nature, which could have led to incomplete data. We had to exclude 59 patients because they did not have $\geq$2 abdominal ultrasonography's during follow-up. This could have led to a selection bias, although the fact that their physician did not order more ultrasonography's probably implies that they estimated the risk of progression as low. In that scenario, our re-

sults would not have changed with inclusion of these patients. Moreover, the retrospective design provides 'real-life' data and a long follow-up time. Lastly, ultrasonography in general has a notoriously large inter-test variability and one can argue whether subtle cases of hepatosplenomegaly were missed by using this technique.[129]

In conclusion, routine abdominal ultrasonography has limited value as a screening tool for progression of ISM. It appears that isolated hepatosplenomegaly has no important prognostic implications in ISM, and changes in liver and/or spleen size alone in fact never herald progression of SM. Moreover, progression of indolent to advanced SM is very rare. Annual follow-up of serum tryptase levels could be used as a first screening tool for the development of hepatosplenomegaly, with additional radiologic studies on indication.

Chapter 4

Drug-related anaphylaxis

4.1 Management around invasive procedures in mastocytosis.

M.A.W. Hermans, N.J.T. Arends, R. Gerth van Wijk, P.M. van Hagen, J.C. Kluin-Nelemans, J.N.G. Oude Elberink, S.G.M.A. Pasmans, P.L.A. van Daele

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Abstract

Objective: Mastocytosis is a chronic hematologic disorder that is characterized by the accumulation of
aberrant mast cells and typically involves the skin and/or bone marrow. Patients with mastocytosis are at increased risk of anaphylaxis. Based on theoretical assumptions, medical procedures requiring general
anesthesia or radiocontrast media are deemed hazardous for patients with mastocytosis. The objective of this article is to provide a comprehensive overview of the actual risk of iatrogenic anaphylaxis and provide recommendations for daily practice.
Data Sources: Various scientific search engines were used (PubMed and Medline).
Study Selections: Because of the paucity of high-level studies on this topic, all available evidence was
considered, including case reports.
Results: Reliable data on the incidence of iatrogenic anaphylaxis in mastocytosis are lacking. However,
although the incidence as reported in (retrospective) cohort studies is higher than in the general population, it is still lower than commonly anticipated, with an incidence of 5.4% in 1 study. Adequate premedication and avoidance of certain physical stimuli can further decrease this risk by 10-fold. The role of drugs as elicitors of anaphylaxis is perhaps overestimated, and physical stimuli are at least as important in inducing release of mast cell mediators.
Conclusion: This article provides practical recommendations for the management of invasive procedures in patients with mastocytosis based on current knowledge of this topic.

Introduction

Mastocytosis is a chronic myeloproliferative disorder of mast cells. It is a rare disease, with an estimated prevalence of 10-13:100,000.[38,130] To establish the diagnosis of systemic mastocytosis (SM), an accumulation of neoplastic mast cells must be detected in the bone marrow, as opposed to cutaneous mastocytosis which is confined to the skin. However, recent experience shows that adult-onset mastocytosis in the skin is associated with systemic mastocytosis in most cases if adequate work-up is performed.[37] The WHO has defined diagnostic criteria for systemic mastocytosis (SM) and various subtypes, ranging from indolent systemic mastocytosis (ISM) to more advanced subtypes.[76] When mast cells are activated, they release large amounts of granule-stored mediators. Consequently, anaphylaxis is a threat for all patients with SM as a result of their high mast cell load, with a lifetime prevalence of up to 50% for adult patients.[131] The most well-known trigger for anaphylaxis in patients with SM is Hymenoptera venom, but many cases of anaphylaxis in SM are idiopathic or the result of a combination of stimuli.[47,132] Conventional allergy tests such as measurement of specific IgE and skin tests for the suspected allergens are often negative. There are various non-IgE-mediated mechanisms that can cause mast cell degranulation, including physical stimuli (temperature change, exercise, strong odors, pressure and friction), and emotional stress. Another often feared elicitor is medication, with anesthetic agents, opiates, and radiocontrast media as the main culprit drugs.[57] In the past decades, several case reports mentioned severe, sometimes fatal, iatrogenic anaphylaxis in mastocytosis, reinforcing the now common notion that this is a serious risk for patients with SM.[133-137]

Based on the aforementioned data and hypotheses, undergoing an invasive procedure often causes anxiety for both patients with SM and the involved professionals. The insecurity is often aggravated by a lack of experience with this rare disease and the diversity of protocols between different hospitals regarding the perioperative management of patients with SM. However, anesthetic techniques have changed and several potentially hazardous drugs are

now obsolete. Furthermore, modern anesthesiologists pay more attention to the general perioperative environment such as temperature and stress.[57,138] Therefore, the actual risk of perioperative anaphylaxis might be overestimated and current protocols could probably be adjusted. In this review, we focus on the actual risk of anaphylaxis around invasive procedures in patients with mastocytosis and give recommendations for prophylactic measures.

Incidence of iatrogenic anaphylaxis in mastocytosis

Several population studies on mastocytosis illustrate the rarity of iatrogenic anaphylaxis. These data are summarized in table 1.

Anesthesia and surgery

The most complete study on this topic reviewed the medical records of 459 adult mastocytosis patients, who underwent 676 anesthetic procedures of which 66 involved general anesthesia.[139] According to the authors (Matito et al.), 8/676 (1.2%) procedures were complicated by a mild reaction and 3 (0.4%) by anaphylaxis. However, the majority were low-risk procedures, namely local anesthesia (76%), epidural anesthesia (11%) or sedation (10%). The risk of mast cell (MC) mediator-related symptoms was considerably higher with general anesthesia, where 4/66 procedures were complicated by a reaction (6%). Anaphylaxis occurred in 2/4 patients, both of whom had not received premedication. Information on the exact procedures is not provided. These rates are higher than the incidence of hypersensitivity reactions due to anesthesia in the general population, which is estimated to be between 1:1250 and 1:18,600 procedures, dependent on the country of investigation.[140] When looking at the whole cohort of Matito et al., the patients who developed any type of reaction less frequently received premedication than asymptomatic patients: 45% versus 87%, respectively. The incidence of anaphylaxis was significantly higher in patients who did not receive any premedication: 5.4% versus 0.4%, respectively.[139] Other risk factors for anaphylaxis were major surgery and a history of anaphylaxis in general, regardless of the trigger of anaphylaxis.[139]

Other, smaller, cohort studies report even lower rates of perioperative anaphylaxis in SM patients; of a combined total of 457 adult patients, only 1 had a history of perioperative anaphylaxis. A reaction to local anesthesia was not reported in any of these studies.[47,48,103,132] Table 1 summarizes the published data from cases. Of course, these numbers can be biased since most of them relied on the medical history of patients as recalled by themselves.

Radiocontrast media

To date, there have been no studies that were specifically designed to identify the risk of anaphylaxis due to radiocontrast media in patients with SM. In the general population, the incidence of mild immediate reactions is 0.5-3%, and the incidence of severe immediate reactions is 0.01-0.04% of all intravenous administrations.[141,142] Indirect evidence shows similarly low numbers of adverse reactions in patients with SM; in four cohort studies encompassing 457 adult patients, radiocontrast-related hypersensitivity reactions were reported in 3 patients, of whom only one experienced anaphylaxis.[47,48,103,132] Data on premedication are incomplete for these patients. Furthermore, a review on fatal anaphylaxis due to radiocontrast media described 34 cases of whom none were known to have mastocytosis. Autopsy of 8 of these fatalities failed to diagnose SM after adequate investigation.[58]

Pregnancy and delivery

Data from 2 cohort studies on pregnancy and delivery in patients with SM are reassuring although the number of patients is small. In the first cohort, labor was uneventful in all 23 women. However, details on the administration of premedication were not provided.[143] The second study describes the course of 45 pregnancies.[144] Five women experienced MC mediator-related symptoms during delivery, consisting of pruritus, erythema or flushing. No-one developed anaphylaxis. Premedication was administered prior to 17 deliveries (38%). One case report described a woman with ISM in whom delivery was complicated by anaphylactic shock despite pretreatment with dexamethasone and diphenhydramine. This patient had previously experienced one ep-

isode of idiopathic anaphylaxis during another pregnancy.[145] For comparison, the risk of anaphylaxis during delivery is 2.7:100,000 deliveries in the general population. However, these reactions are virtually always due to IgE-mediated drug allergies.[146]

Table 1. Summary of published data on iatrogenic anaphylaxis in adult patients with mastocytosis.

	Anaphylaxis due to general anesthesia	Mild reaction due to general anesthesia	Anaphylaxis due to radiocontrast media	Mild reaction due to radiocontrast media	Anaphylaxis related to delivery	Other cause of iatrogenic MC mediator related symptoms
González de Olano et al. 2007[7]	1/163	0/163	0/163	1/163	N/A	NSAID (4) Bèta lactam (2) Streptomycin (1) Phenylephrine (1) Codeine (1)
Matito et al. 2007[15]	2/66	1/66	N/A	N/A	N/A	Epidural (3/76)* Sedation (1/67) Local anesthesia (4/515)
Brockow et al. 2008[6]	0/74	N/A	2/74	N/A	N/A	Local anesthesia (1) NSAID (1) Codeine (1) Amoxicillin (1)
Gülen et al. 2013[17]	0/84	0/84	0/84	0/84	N/A	NSAID (2)
Hermans et al. 2016[18]	0/136	0/136	1/136	0/136	N/A	NSAID (2)
Case reports[10-14,24]	3	N/A	1	N/A	1	Percutaneous coronary intervention (1)
Worobec et al. 2000[60]	-	-	-	-	0/11	N/A
Matito et al. 2011[23]	-	-	-	-	0/45†	N/A
Ciach et al. 2016[22]	-	-	-	-	0/23	N/A
Total‡	3/523	1/449	3/457	1/383	0/79	26/602

N/A: not available; MC: mast cell; NSAID: non-steroidal anti-inflammatory drug.
* 1 patient anaphylaxis, 2 patients mild reaction (flushing, erythema or hives).
† 5/45 patients had mild MC mediator symptoms (pruritus, generalized exanthema, flushing).
‡ Excluding case reports.

Theoretical background

Known triggers of mast cell activation

As previously stated, many substances other than IgE can trigger release of MC mediators. Interestingly, mast cells in the skin and airways of patients with mastocytosis are not more reactive compared with patients with asthma or healthy controls.[147] Specific drugs that are known to induce histamine release are codeine, morphine, benzylisoquionolins (e.g. mivacurium, atracu-

rium) and some antibiotics (e.g. vancomycin, polymyxin B).[148] The potential of codeine and morphine to induce MC degranulation was proven extensively both *in vitro* as *in vivo*.[59] Most other opiates do not have this effect when tested *in vitro*, revealing a certain class effect.[149,150] For muscle relaxants, there also appears to be a large variety in their ability to induce release of MC mediators, with succinylcholine and cis-atracurium appearing as the most safe drugs in this context.[151,152] Radiocontrast media could theoretically trigger MC degranulation via multiple mechanisms, for instance the direct effect of their high osmolality on the cell membrane, or nonspecific binding of contrast molecules to membrane receptors and components of the complement system.[58] However, *in vitro* tests did not show degranulation after stimulation of human MCs with radiocontrast media.[153] Unfortunately, most of these assumptions are based on *in vitro* research, which is notoriously complicated for mast cells since they are continually interacting with adjacent cells *in vivo*.[26]

Next to medication, several physical stimuli might induce MC mediator release during invasive medical procedures, or act as costimulatory factors.[47] Among these are friction, pressure, temperature changes, and emotional stress. The reaction to physical stimuli can vary between patients but severe anaphylaxis due to physical stimuli alone is rare. Mostly they serve as cofactors in combination with other stimuli.[47] Moreover, MC have a great phenotypic variation depending on the tissue they reside in. The composition of released mediators may vary accordingly, and also ranges depending on the kind of stimulus. For instance, procedures involving the gastrointestinal tract might theoretically be more prone to MC degranulation since these organs contain many MC.[154]

Table 2. High-risk procedures that form indications for premedication: any of the following criteria.

Characteristics of procedure
General anesthesia
Major surgery
Gastrointestinal or cardiac surgery*
Patient characteristics
Previous MC mediator-related symptoms during procedure
History of anaphylaxis (regardless of trigger)
Use of beta-blockers†, ACE-inhibitors‡, NSAIDs‡
Severe mastocytic infiltration of the skin

* risk factor on theoretical grounds; no clinical evidence.
† beta-blockers may attenuate the effect of epinephrine in case of anaphylaxis but are no risk factor in itself.
‡ ACE-inhibitors and NSAIDs can augment an anaphylactic reaction as cofactors.

Risk factors for anaphylaxis

Previous cohort studies have shown that anaphylaxis is more common in patients with mastocytosis who have a history of idiopathic anaphylaxis, and particularly those with ISM without skin involvement.[47,50,77,103] Furthermore, higher total serum IgE levels and older age are associated with an increased risk of anaphylaxis.[50,57] Of note, many studies on risk factors for anaphylaxis focus on Hymenoptera venom-related anaphylaxis and it is not clear whether these data can be extrapolated to iatrogenic anaphylaxis in patients with mastocytosis. Moreover, most studies included patients without mastocytosis which is an essentially different population. For instance, increased serum tryptase levels are associated with a higher risk of anaphylaxis in patients without mastocytosis [155-157], whereas a bell shaped curve is described in mastocytosis. [158] In the latter, patients with a serum tryptase level between circa 12 and 40 µg/L had the highest risk of Hymenoptera venom related anaphylaxis, and this risk decreased with a further increase of serum tryptase levels.[158] A higher mast cell load might thus be "protective" of anaphylaxis, possible through a favorable antigen:mast cell ratio, however this is purely speculative.

Rationale for premedication

Since the available data on the risk of iatrogenic anaphylaxis are conflicting, the value of premedication remains unclear. Although the overall risk of severe anaphylaxis appears to be low, a distinction must be made between low- and high-risk procedures (table 2). For the latter, it seems reasonable to give premedication. However, it is less evident which drugs should be used as prophylaxis. The most important mediators in acute anaphylaxis are histamine, leukotrienes, prostaglandins, proteoglycans, TNF-α and platelet activating factor.[159] Therefore, premedication should consist of drugs that block these mediators. Histamine receptor antagonists only provide relief of cutaneous symptoms like erythema and pruritus, and do not protect against anaphylaxis.[160] There is additional evidence showing the synergistic effect of H2-receptor antagonist on the pharmacokinetics of H1-receptor antagonists, arguing for combining these two.[161] Leukotriene antagonists appear less effective in attenuating mast cell mediator related symptoms, although randomized trials in patients with mastocytosis are lacking.[162] Furthermore, benzodiazepines are valuable to remove the trigger of emotional stress and thereby may reduce the risk of perioperative anaphylaxis in SM patients.[139]

Despite the paucity of randomized clinical studies on this topic, corticosteroids are widely used in protocols for the prophylaxis of acute anaphylaxis. There are several well-designed *in vitro* studies that prove that corticosteroids have an acute effect on MC degranulation and activation, probably through membrane-bound glucocorticoid receptors.[59,163-165] Studies that have used skin tests with allergens as a model for MC reactivity have contradictory results, possibly reflecting the difference in duration of corticosteroid use, since only corticosteroid use of short duration suppressed skin test reactivity.[166,167] Lastly, one randomized clinical trial performed in the '90s compared prophylaxis with 32 mg methylprednisolone at 12 and 2 hours prior to the administration of radiocontrast media, with placebo. Methylprednisolone reduced the risk of hypersensitivity reactions from 4.9% to 1.7%.[168] Although the total number of reactions was low and both studies did not include mastocytosis patients,

these data suggest some benefit from the inclusion of corticosteroids in a premedication regime. Hence, the administration of corticosteroids shortly before a procedure might attenuate MC degranulation, and makes more sense than giving corticosteroids only once a patient is anaphylactic and extensive degranulation has already occurred.

Table 3. Safety of perioperative drugs for patients with mastocytosis.

	I.V. hypnotics	Inhaled hypnotics	Local anesthetics*	Neuromuscular blocking agents
Recommended	Etomidate Propofol Ketamine	Desflurane Isoflurane Nitrous oxide Sevoflurane	Amide-type (e.g. lidocaine)	Succinylcholine Cis-atracurium Pancuronium Vecuronium
Unclear	Thiopental		Ester-type (e.g. procaine)	Rocuronium
Discouraged				Rapacuronium Atracurium Mivacurium
	Analgesics	**Antiseptics**	**Plasma substitutes**	**Miscellaneous agents**
Recommended	Fentanyl	Chlor-hexidine	Albumin	Atropine
	Sufentanil	Povidone iodide	Gelatin	Ondansetron
	Remifentanil Alfentanil Acetaminophen			Oxytocin
Unclear	Morphine‡ NSAID's†		HES starch	Protamine
Discouraged	Codeine Nefonam			Aprotinin (fibrin glue)

* Severe systemic reactions to local anesthetics are very rare, and often IgE-related.
† NSAID = nonsteroidal anti-inflammatory agents. Avoid if not used before.
‡ Titrate slowly; rapid infusion can aggravate MC mediator release.

Previously published protocols for perioperative prophylaxis

In brief, there are few published practical guidelines on prophylaxis around invasive procedures in patients with mastocytosis. An EAACI position paper on drug hypersensitivity in mast cell disease states that the evidence on the risk of iatrogenic anaphylaxis is low, as well as the evidence for premedication. Mainly based on expert opinion, the authors recommend to consider the administration of H1 antagonists, benzodiazepines and corticosteroids prior to invasive procedures. Also, the importance of cofactors such as temperature changes and pressure is stressed. [57] Other published reviews make

similar statements but often do not go further than generalities, without providing tangible advice regarding which medication and dosages to use as premedication. Lists of drugs that should be avoided in mastocytosis are based on theoretical assumptions and some case reports.[154,169, 170] In order to create a protocol which is as evidence-based as possible, well-established guidelines for prevention of radiocontrast in general were taken into account in writing these recommendations.[171-173]

Practical recommendations

Based on the aforementioned considerations and published expert reviews and protocols on this topic [57,154,160,169,170,172-174], we have composed structured recommendations for lowering the risk of anaphylaxis around medical procedures in patients with all forms of mastocytosis.

General considerations

First, a risk analysis should be made to determine whether premedication is indicated. Risk factors that could necessitate premedication are summarized in table 2, but this list is not complete and individual considerations are necessary for each patient. We recommend that a plan of action is made before the procedure by a multidisciplinary team consisting of a specialist in the field of mastocytosis, anesthesiologist, and the physician performing the procedure. It is preferable to involve the patient in this stage to reduce any insecurities on their side. A detailed survey on previous anaphylaxis and known drug allergies is of paramount importance in the prevention of iatrogenic anaphylaxis. A comprehensive work-up for specific drug allergies should only be considered if a patient has previously experienced drug-related anaphylaxis, since patients with mastocytosis can also develop 'ordinary' IgE-mediated allergies. When drug sensitization testing is not possible, drugs that have caused adverse reactions in the past need to be avoided. There is no current role for preoperative drug sensitization testing in the absence of previous allergic reactions.[170] Non-steroidal anti-inflammatory drugs (NSAID's) should be avoided if the patient has never used them before. On the contrary, if

NSAID's have been used before without any problems, they can be continued. As outlined before, several physical stimuli can induce MC degranulation, and all members of the treatment team need to be aware of this. Ambulatory surgery is also possible in patients with mastocytosis, since premedication can be given orally in most cases.

Prophylactic treatment prior to high-risk procedures.

Here, we have outlined our protocol for the management around high-risk procedures in patients with mastocytosis. Specific medications and dosages can vary between countries. Also, physicians can choose to partially follow the protocol, for example to only give a histamine receptor antagonist prior to low-risk procedures.

Preoperative

- Provide benzodiazepine to reduce anxiety 1-2 hours before procedure.
- Corticosteroids:
    - Twelve hours and 2 hours before procedure: 0.5 mg/kg prednisolone or equivalent for oral administration, with a maximum dose of 60 mg.

      -or-
    - In case of emergency procedure: 200 mg hydrocortisone intravenously.
- Histamine receptor antagonists:
    - 2 hours before procedure: 10 mg levocetirizine or equivalent fast-working H1-receptor antagonist orally and 300 mg raniditine or equivalent H2-receptor antagonist orally

      -or-
    - 15 minutes before procedure: 2 mg clemastine or equivalent H1-receptor antagonist intravenously and 300 mg raniditine or equivalent H2-receptor antagonist intravenously.

Perioperative

- Close monitoring and anesthesiologist present in the room.
- Limit changes in room temperature, both increase or decrease.
- Avoid pressure or friction of the skin as much as possible , especially in patients with extensive cutaneous mastocytosis.
- Avoid drugs as noted in table 3.
- Keep epinephrine ready to use (adjust to patient weight).

Postoperative

- Avoid drugs as noted in table 3.
- General considerations for avoidance of physical stimuli still need to be adhered to.

Local anesthesia

Local anesthesia is generally safe in mastocytosis patients. Rare cases of anaphylactoid reactions associated with local anesthetic procedures were probably the result of physical stimuli or IgE-mediated allergies. Moreover, vasovagal collapse is sometimes mistaken for anaphylaxis. Premedication is not advised in procedures where only local anesthesia or epidural anesthesia is used.

Radiological contrast media

Iodized radiocontrast media have a higher risk of anaphylaxis than gadolinium, although there are some (rare) cases of severe anaphylaxis after gadolinium administration in the general population.[171] There is no rationale for the avoidance of contrast media. Premedication is only indicated when a patient has previously experienced anaphylaxis due to radiocontrast media, or in patients whom are estimated to have a high risk of radiocontrast induced anaphylaxis (table 2).

Cardiologic interventions

Mastocytosis can first present with cardiac symptoms such as unexplained

syncope or Kounis syndrome (coronary spasms). Since mast cells are constitutively present in the heart, a cardiologic intervention might theoretically pose a risk for anaphylaxis. To date, merely one case report was published of anaphylactic shock during percutaneous coronary intervention in a patient who had a history of unexplained syncope.[175] Of course, the administration of radiocontrast media can trigger anaphylaxis in itself. We therefore suggest to use the same considerations for prophylaxis before percutaneous cardiologic interventions, as for radiocontrast media. However, it must be noted that this suggestion is mostly based on theoretical assumptions as randomized studies are lacking.

Delivery

Based on the available data, premedication before an uncomplicated delivery is not strictly necessary. However, it can be considered for patients who have experienced anaphylaxis unrelated to delivery before, especially for those with previous idiopathic or iatrogenic anaphylaxis. Premedication is also indicated for deliveries for which general anesthesia is required. No teratogenicity was described for H1-antagonists, although sedative H1-antagonists can induce sedation in the newborn child when used directly prior to the delivery. Cetirizine is the preferred H1-antagonist in pregnant women. Ranitidine can also be used safely during pregnancy and labour.[176]

Children

Prophylaxis of anaphylaxis in children with mastocytosis is outside the scope of this review. Although the incidence of anaphylaxis is much lower in pediatric mastocytosis than in adults, iatrogenic anaphylaxis is reported in children and the same recommendations for premedication probably apply, with dose reductions if necessary.

Acute treatment of anaphylaxis

In the case of anaphylaxis, the patient should be treated according to current guidelines for anaphylaxis.[160] The first step is to remove the trigger. Next,

timely administration of epinephrine is the single-most important lifesaver in this context. Of note, the dosage of epinephrine for anaphylaxis is lower than in the setting of a cardiac arrest (0.5 mg if body weight >60 kg), and it should be given intramuscularly in the mid-outer thigh. Epinephrine i.m. can be repeated after 5-10 minutes when the first dose is not effective. If intramuscular epinephrine appears ineffective after 2-3 doses, continuous intravenous administration can be considered.[160] After stabilizing the patient, histamine receptor antagonists are mainly effective for the relief of cutaneous symptoms.[161] Although the evidence is weak, corticosteroids might attenuate protracted anaphylaxis and can be administered once all first-line treatment steps are completed.[160,177]

Conclusion

The risk of iatrogenic anaphylaxis in mastocytosis patients is generally lower than most physicians anticipate. However, the risk is increased compared with the general population, and anaphylaxis might be more severe in mastocytosis patients. Adequate perioperative management reduced the incidence of anaphylaxis from 5.4% to 0.4% in one study, securing the indicated treatment. A structural, patient-tailored risk assessment and subsequent therapeutic plan is pivotal in this context. The role of drugs as elicitors of anaphylaxis is probably overestimated, and physical stimuli are at least as important in inducing release of mast cell mediators.

4.2 Low frequency of acetyl salicylic acid hypersensitivity in mastocytosis: the results of a double-blind, placebo-controlled challenge study.

M.AW. Hermans, S.Q.A. van der Vet, P.M. van Hagen, R. Gerth van Wijk, P.L.A. van Daele

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Abstract

Background: Patients with mastocytosis are at increased risk of anaphylaxis. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is often discouraged because of this reason. However, the actual prevalence and severity of NSAID-related hypersensitivity among patients with mastocytosis is unknown.

Methods: A double-blind, placebo-controlled acetylsalicylic acid (ASA) challenge up to a cumulative dose of 520 mg was performed among adult patients with mastocytosis. In addition, a retrospective search of the entire outpatient cohort was performed to obtain 'real life' data on NSAID hypersensitivity.

Results: Fifty patients underwent an ASA challenge. Seventy percent had indolent systemic mastocytosis, 18% mastocytosis in the skin, 12% advanced mastocytosis. The ASA challenge was positive in 1 patient who developed urticaria. The additional retrospective chart review revealed that 8 out of 191 patients had a history of NSAID-related hypersensitivity reaction(s), of whom 3 reported severe systemic reactions. All 8 patients had already experienced NSAID-related hypersensitivity reactions before mastocytosis was diagnosed.

Conclusions: The frequency of ASA hypersensitivity as determined by a prospective challenge study was 2%, and 4.1% in a retrospective chart review of 191 patients with mastocytosis. NSAIDs can be administered safely to most patients with mastocytosis. Extra caution should be taken in patients with a history of hypersensitivity reactions to other drugs, or traditional risk factors for NSAID hypersensitivity.

Introduction

Mastocytosis is a disease in which aberrant mast cells accumulate. The WHO recognises different subtypes of SM [33,76]. The prevalence of anaphylaxis is higher in patients with mastocytosis compared to healthy persons [178,179]. A wide variety of stimuli can trigger mast cell degranulation and thereby lead to anaphylaxis [7]. Historically, the use of certain medications that could theoretically trigger mast cell degranulation is discouraged in patients with mastocytosis. Among these are radiocontrast media, general anaesthetics, opioid analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) [178]. For general anaesthetics and radiocontrast media, case reports on severe (and sometimes lethal) anaphylaxis in patients with mastocytosis are available, although the absolute risk still appears low [133,180,181].

The prevalence and severity of anaphylaxis due to NSAIDs in patients with mastocytosis is actually not known. Anxiety for NSAID related hypersensitivity reactions leads to confusion among both physicians and patients, and different advices between practices. The use of NSAIDs is therefore often avoided, resulting in the risk of mistreatment of patients with mastocytosis for several reasons. Firstly, these patients are at increased risk of cardiovascular morbidity, which often necessitates acetylsalicylic acid (ASA) for secondary prevention [61,179]. Secondly, ASA is a well-known treatment for flushing in mastocytosis [182,183]. Thirdly, patients with mastocytosis relatively often suffer from various types of pain for which analgesics might be necessary [79,184]. Currently, they are often advised to only take acetaminophen which is not always sufficient.

In our practice, we noted that many patients used NSAIDs uneventfully, until they received were diagnosed to have mastocytosis. Furthermore, previously performed NSAID challenges were always negative. We therefore hypothesized that the frequency and severity of NSAID related hypersensitivity is overestimated in patients with mastocytosis. For drug hypersensitivity in general, drug challenge tests are the gold standard diagnostic procedure[185]. ASA is often used to test for general NSAID hypersensitivity because of its strong COX-1 inhibiting properties[186]. Therefore, we designed a study using standardised

drug challenge tests with ASA to investigate the exact prevalence and severity of NSAID hypersensitivity reactions in patients with mastocytosis.

Methods

Patient eligibility

Patients were recruited from the mastocytosis outpatient clinic of the Erasmus University Medical center. All adult patients with biopsy-proven cutaneous or systemic mastocytosis were eligible. Patients were excluded if they had a history of a prior NSAID-related hypersensitivity reaction(s), uncontrolled asthma, rhinosinusitis, nasal polyps, active pregnancy, high dosage of beta blocking drugs (equivalent to ≥ 100 mg. of metoprolol), when they were not able to stop antihistamines or prednisolone, or were not deemed capable of handling possible delayed anaphylactic reactions at home. Pre-existent mast cell mediator-related symptoms such as pruritus or flushing were not considered to be exclusion criteria in order to represent the real life situation at an outpatient clinic. Moreover, flushing can be an indication for ASA.

Study protocol

All patients underwent a double-blind, placebo-controlled ASA challenge in a randomized order. The minimum interval between the two test days was 14 days. The study medication was provided in a blinded fashion by the pharmacy of the Erasmus MC. Patients had to stop H1-antagonists and leukotriene antagonists for three days prior to the drug challenge. The challenge took place at the Allergy outpatient clinic. Patients received three incremental doses of ASA of 40 mg., 80 mg., and 400 mg. (or matched placebo tablets), leading to a cumulative dose of 520 mg. The interval between each dose was one hour and patients were observed for an additional two hours after the administration of the third dose.

Mast cell mediator-related symptoms were systematically scored before the start of each drug challenge and after one, two and four hours. We used an adapted form of the scoring system for food challenges as proposed by Grabenhenrich et al.[187] This form scores symptoms according to organ system

and severity and is in our practice routinely used for both food and drug challenges. In addition, numerous rating scale (NRS) score were obtained for mast cell mediator-related symptoms such as pruritus and headache. An increase of 3 points in this scale during the challenge was considered significant. All challenges were conducted and assessed by MH and/or SdV. In cases of doubt, a second investigator (RGvW or PvD) was consulted to assess the symptoms. Deblinding of the investigators and patients took place 24 hours after all 50 patients completed both challenge days.

Outcomes and definitions

The ASA challenge was considered positive when a patient developed objective mast cell mediator-related symptoms within 12 hours after the administration of the third dose on the day they received the verum, and had no symptoms on the placebo day. The challenge was considered negative when a patient developed no symptoms on the verum day, regardless of any symptoms on the placebo day.

The WHO criteria were used to define the subtype of mastocytosis [76]. Patients with maculopapular cutaneous mastocytosis (MPCM) who never underwent bone marrow biopsy, or had negative bone marrow investigation, were categorized as mastocytosis in the skin (MIS). An atopic background was defined as a history of atopic dermatitis, asthma, rhinoconjunctivitis, and/or positive specific IgE for inhalation or food allergens. Next to atopy, other traditional risk factors for NSAID hypersensitivy were defined as the presence of: asthma, nasal polyps, chronic rhinosinusitis. Eosinophilia was defined as an absolute eosinophil count of > $500x10^6$ in peripheral blood.

Additional retrospective cohort study

Next to the prospective challenge study, we retrospectively searched the electronic patient records of all adult patients who visited the mastocytosis centre from January 2009 until January 2017 and who fulfilled the criteria for mastocytosis in the skin (MIS), cutaneous or systemic mastocytosis (SM). Patients who already participated in the challenge study were excluded from

the retrospective cohort. Patients with a history of NSAID-related hypersensitivity reactions, or patients who had proven NSAID tolerance prior to the start of this study, were subsequently contacted to obtain further clinical details. NSAID tolerance was considered as proven when a drug challenge was negative or when (accidental) NSAID ingestion was uneventful after the diagnosis of mastocytosis was made. The characteristics of the patients with and without NSAID tolerance from this retrospective cohort were compared to identify possible differences.

Ethical considerations

This trial was performed according to the latest Helsinki guidelines. The study was approved by the local medical ethics committee. All participants provided written informed consent. The trial was registered in the EudraCT database, number 2015-004604-37.

Statistical analysis

We used IBM SPSS 21 for all analyses. Patient characteristics were noted as median with interquartile range (IQR) for continuous variables and as the number with percentage for dichotomous variables. To calculate potential differences between the groups with and without NSAID hypersensitivity, the Mann Whitney U test was used for continuous variables and the chi square test for dichotomous variables.

Power calculation

The prevalence of NSAID-related hypersensitivity reactions in the general population is less than 1%. We hypothesized that the risk in patients with systemic mastocytosis is only marginally higher than in the general population. With an estimated frequency of allergic reactions of 4% in our study population, inclusion of 50 subjects in total will lead to a 95% confidence interval of 1-13%. The estimated frequency was based on self-reported reactions by patients in the Erasmus MC cohort combined with circumstantial data of other cohort studies on mastocytosis (see Discussion section for references).

Results

Study population

At the moment of inclusion in April 2017, 173 patients were considered eligible to participate in the trial (figure 1), and 58 patients signed the informed consent. After inclusion, 8 patients dropped out before the ASA challenge was performed completely. One of these patients was excluded after the first challenge day because she started to use prednisolone for arthritis which was not related to the trial. Two patients experienced anaphylactoid reactions related to their mastocytosis within days after cessation of the antihistamines. The other patients withdrew consent. The inclusion process was stopped after 50 subjects completed both days of the challenge. The final study population thus consisted of 50 patients. The median age was 55 years, and most participants had indolent SM (table 1).

Figure 1. Flow chart of the inclusion process.

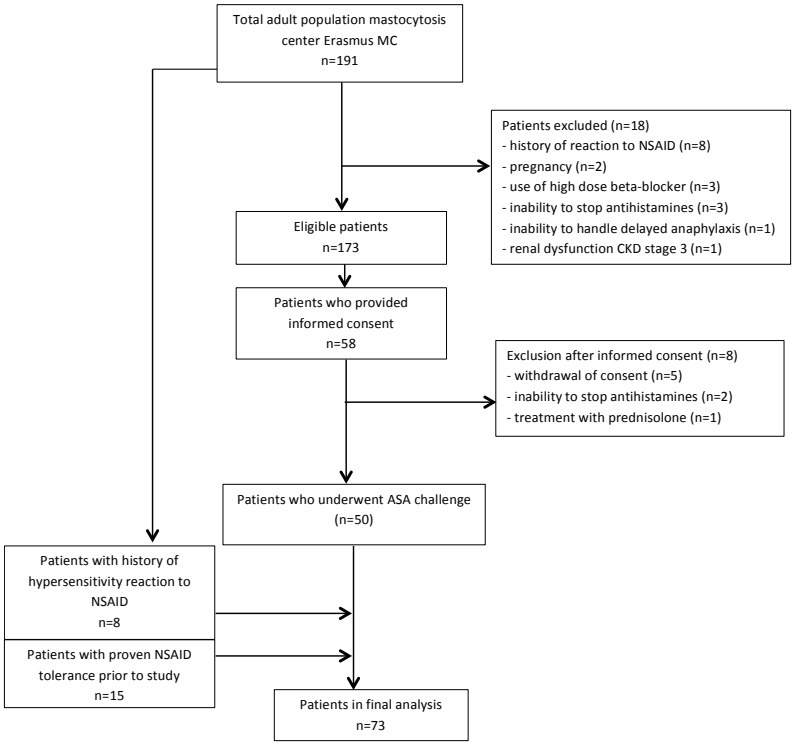

Table 1. Baseline characteristics study population (n=50).	
Age in years, median (IQR)	55 (16)
Male, n (%)	16 (32)
Subtype, n (%)	
• MIS	9 (18)
• ISM	
with skin lesions	26 (52)
without skin lesions	9 (18)
• SSM	3 (6)
• SM-AHN	2 (4)
• ASM	1 (2)
Serum tryptase level at diagnosis in μg/l, median (SD)	25.0 (17.8)
Atopic background, n (%)	13 (26)
Eosinophilia, n (%)	2 (4)
Previous hypersensitivity reaction to any drug, n (%)	5 (10)
Previous anaphylaxis due to any trigger, n (%)	23 (46)
• Wasp	7
• Unknown	7
• Physical stimuli	4
• Other drugs*	5
• Miscellaneous†	6

MIS: mastocytosis in the skin; ISM: indolent systemic mastocytosis; SSM: smouldering systemic mastocytosis; SM-AHN: systemic mastocytosis with associated hematological neoplasm; ASM: agressive systemic mastocytosis.
*Other drugs: proton pump inhibitor (1x), morphine (2x), penicillin (1x), and codeine (1x).
†Miscellaneous triggers: horsefly (n=2), jellyfish sting (n=1), fire ant (n=1), anesthesia (n=1), codeine (n=1).

Results of ASA challenge

The challenge was positive in one patient (2%), who developed an urticarial rash 4 hours after ingestion of the third dose of ASA, which corresponds with a cumulative dose of 520 mg. The rash subsided after she took 10 mg. of hydroxyzine. This patient had smouldering SM based on a serum tryptase level of ≥ 200 μg/L and hepatosplenomegaly. She had never used NSAIDs before. She had previously developed a rash after the administration of radiocontrast media and reported an increase in mast cell mediator-related symptoms after the consumption of alcoholic beverages and histamine rich food.

Three other patients reported subjective symptoms on the day of the verum but not on the day they received placebo. These symptoms consisted of mild

flushing in 1 patient, generalized pruritus in 1 patient, and lightheadedness in 1 patient. The flushing was not considered as a positive challenge because it occurred after the second dose of 80 mg. and subsided spontaneously despite the next increasing dose of ASA was administered according to protocol. Moreover, this patient has spontaneous flushes multiple times a week. Similarly, the patient with pruritus already had pruritus before the start of the challenge and the NRS score increased by 2 points throughout the day of the challenge which was below the pre-specified threshold of 3 points (see Methods section). The last patient experienced lightheadedness 15 minutes after the ingestion of the third dose of ASA, but had no other mast cell mediator-related symptoms and stable vital parameters. The serum tryptase level did not increase as compared to a baseline measurement. Seven patients had a reaction on the placebo day, of whom 1 had objective macular erythema on the arms and trunk, and the other 6 patients had subjective symptoms. Four patients who had a reaction to placebo already had mast cell mediator-related symptoms at the start of the challenge, consisting of pruritus and/or flushing.

Patients with a history of NSAID-related reactions

In addition to the challenge study, we retrospectively searched the electronic records of all adult patients with mastocytosis that visited the Erasmus MC from 2009 until 2017. Out of a total of 191 patients, 8 patients had an annotation of 'NSAID allergy' in their medical record. This results in a prevalence of self-reported NSAID-related hypersensitivity of 4.1% in our entire cohort. Fifteen patients had proven NSAID tolerance prior to this study. Table 2 summarizes the clinical characteristics of these patients.

All patients had experienced NSAID-related reactions before they received the diagnosis of mastocytosis. The most frequent symptoms were angioedema, erythema and hypotension. Three patients required treatment in an emergency department. Of the patients who could reliably recall their reaction at the time of questioning, everyone experienced a reaction within 2 hours after the ingestion of the NSAID. Patient number 5 developed a reaction after

the combination of naproxen and a wasp sting. Hymenoptera sensitization could not be confirmed. Although it is likely that Hymenoptera was the main culprit and naproxen acted as a cofactor, the patient was labelled as 'NSAID intolerant' because of the severity of the reaction and the risk of aggravation of future reactions with the use of NSAIDs. Patient number 3 later had a drug challenge with celecoxib which was negative. Patient number 6 and 8 both later had a negative drug challenge with naproxen, which excludes a general non-specific NSAID hypersensitivity. Notably, seven patients (87.5%) reported mast cell mediator-related reactions to other physical stimuli. These reactions ranged from flushing or gastrointestinal symptoms to anaphylaxis.

Table 2. Characteristics of patients with hypersensitivity reaction(s) to NSAIDs.

	Age at diagnosis (years)	Sex	Subtype *	Mastocytosis in the skin	Serum tryptase at diagnosis (µg/l)	Atopy	Type of NSAID	Timing of reaction	Symptoms of reaction	Reaction to other triggers
1	41	F	MIS	TMEP	8.6	Yes	ASA 80 mg.	2 hours	Angioedema	Penicillin, possibly lidocaine
2	72	M	ISM	No	20.0	No	Ibuprofen Diclofenac†	unknown	Generalized pruritus, blurry vision	-
3	51	F	ISM	MPCM	125.0	Yes	Ibuprofen Diclofenac†	5 min	Angioedema, palpitations, collapse‡	Heat
4	62	F	ISM	MPCM	118.0	No	Diclofenac	unknown	Angioedema	Alcohol consumption
5	42	M	ISM	MPCM	31.4	No	Naproxen	10 min	Erythema, stridor, hypotension‡	Alcohol consumption, wasp sting, temperature changes (cold)
6	40	M	ISM	No	24.7	Yes	Acetamino-phen	5 min	Diffuse erythema	Strong odors or dust
7	48	F	ISM	MPCM	43.5		Naproxen	5 min	Diffuse erythema‡	-
8	68	F	ISM	MPCM	17.8	No	Diclofenac	20 min	Hypotension, collapse‡	Iodated contrast media

MIS: mastocytosis in the skin. TMEP: teleangiectasia macularis eruptiva perstans. MPCM: maculopapular cutaneous mastocytosis. ASA: acetylsalicylic acid. NSAID: nonsteroidal anti-inflammatory drug

*Bone marrow biopsy was performed in all patients.

† Two separate reactions at different occurrences.

‡ Treatment at emergency department required.

Characteristics associated with NSAID hypersensitivity

Since only one patient had a positive ASA challenge, the data from the prospective study could not be used to reliably identify any potential clinical characteristics that are associated with NSAID hypersensitivity. Therefore, data from the retrospective cohort were analyzed for this purpose (table 3).

Overall, patients with NSAID tolerance appear to have more daily mast cell mediator-related symptoms such as flushing, cognitive problems, fatigue and pruritus. Only the latter was statistically significant (p 0.021, chi square test), probably due to the small numbers of patients. Patients with NSAID hypersensitivity reported more reactions to other drugs, although this difference did not reach statistical significance (p 0.063). The same accounted for peripheral blood eosinophilia (p 0.181), osteoporosis (p 0.186) and alcohol intolerance (p 0.308). Strikingly, traditional risk factors for NSAID hypersensitivity in the general population such as atopy, asthma or rhinitis were not more frequent in the NSAID hypersensitivity group of our cohort. Neither were there any relevant differences in age, sex, serum tryptase levels, or skin involvement of mastocytosis.

Table 3. Comparison of clinical characteristics of patients with and without NSAID hypersensitivity of mastocytosis cohort EMC, as proven by drug challenges.

	No NSAID hypersensitivity (n=64)	NSAID hypersensitivity (n=9)	p-value
Age, median (IQR)	55 (10)	51 (9)	NS
Male sex, n (%)	22 (34.4)	3 (33.3)	NS
Subtype, n (%)			NS
- **MIS**	14 (21.9)*	1 (11.1)†	
- **ISM**	45 (70.3)	7 (77.8)	
- **SSM**	2 (3.1)	1 (11.1)	
- **SM-AHN**	2 (3.1)	0	
- **ASM**	1 (1.6)	0	
Presence of skin mastocytosis, n (%)	50 (78.1)	6 (66.6)	NS
Serum tryptase at diagnosis, median (IQR)	25 (7.8)	31.4 (11.4)	NS
History of anaphylaxis, n (%)	27 (42.2)	5 (55.6)	NS
Pruritus, n (%)§	50 (78.1)	1 (11.1)	NS
Flushing, n (%)§	27 (42.2)	2 (22.2)	NS
Dyspepsia, n (%)§	10 (15.6)	2 (22.2)	NS
Diarrhea, n (%)§	15 (23.4)	0	NS
Fatigue, n (%)§	37 (57.8)	2 (22.2)	0.04
Subjective cognitive problems, n (%)	22 (34.4)	0	0.045
Osteoporosis, n (%)	8 (12.5)	3 (33.3)	NS
Eosinophilia, n (%)	4 (6.3)	3 (33.3)	0.01
Atopy, n (%)	17 (26.6)	3 (33.3)	NS
History of hypersensitivity reaction to other drugs, n (%)‡	5 (7.8)	4 (44.4)	0.002
Alcohol intolerance, n (%)‖	29/50 (58)	4/5 (80)	NS
MC mediator related reaction to physical triggers, n (%)¶	48 (75)	5/6 (83.3)	NS

*bone marrow investigation negative in 2 patients, other 12 never underwent bone marrow punction

§ Symptom present ≥3 days per week.

† Bone marrow investigation negative for bone marrow mastocytosis.

‡ See text for further explanation.

‖ Not known for all patients because some patients never consume alcohol.

¶ Physical triggers: heat, cold, stress, exercise, alcohol consumption.

Discussion

This is the first double-blind, placebo-controlled challenge study to investigate the prevalence and severity of ASA hypersensitivity among patients with mastocytosis. Only 1/50 participants (2%) had a positive ASA challenge, consisting of an urticarial rash. Three other patients had subjective symptoms to ASA. The characteristics of the study population were overall representative of a patient cohort in a tertiary centre for mastocytosis, except for a relatively low number of male participants [77,83,103].

However, the exclusion of patients with known risk factors for NSAID hypersensitivity might have led to a selection bias. Therefore, we performed an additional retrospective analysis of our entire cohort of 191 patients. This resulted in a prevalence of self-reported NSAID-related hypersensitivity of 4.1%. Importantly, all patients with NSAID hypersensitivity experienced one or more reactions before the diagnosis of mastocytosis was established. Although interpretation of any differences between the patients with and without NSAID hypersensitivity is difficult due to the small patient numbers, it appears from the retrospective cohort that patients with NSAID hypersensitivity more often experienced hypersensitivity reactions to other drugs and/or alcohol. It must be noted that three patients reported a hypersensitivity reaction to amoxicillin, which is the third most reported culprit for drug-related reactions in The Netherlands. We cannot exclude that the relationship between amoxicillin and the reported reactions was based on coincidence. Another notable difference is the higher prevalence of mast cell mediator-related symptoms among NSAID tolerant patients. Possibly, this difference represents the clinical practice, because patients with symptoms such as flushing are more often in need for ASA and therefore were more likely to undergo an NSAID challenge out of medical necessity. A causal explanation seems unlikely.

Interestingly, 2/8 patients with a history of NSAID-related hypersensitivity reactions later had negative un-blinded challenges with another NSAID. This can be explained in multiple ways: they might have a specific, IgE mediated, allergy to the culprit NSAID. Another, more likely, explanation is the fact that NSAIDs can be a cofactor to augment anaphylaxis [47,188]. The current trial does not provide prospective data on the role of NSAIDs as a cofactor in patients with mastocytosis. Also, although the use of ASA as a model for general NSAID hypersensitivity is widely accepted, a specific allergy for one type of NSAID is potentially missed with this approach. Moreover, the currently presented data cannot be extrapolated to patients with traditional risk factors for NSAID hypersensitivity, such as asthma, nasal polyposis or atopic constitution. However, most patients with mastocytosis do not have such risk factors [132], thus

most patients would fulfill the currently used inclusion criteria. Lastly, a possible caveat of drug challenges in patients with mastocytosis is the fact that many of them already have mast cell mediator-related symptoms on a daily basis, especially since anti-mediator medications need to be interrupted prior to a challenge. By using NRS scores, we tried to score these symptoms as objectively as possible, however, some degree of bias in the assessment of challenge studies cannot be excluded in this patient category.

The prevalence of NSAID hypersensitivity in our cohort of patients with mastocytosis is only slightly higher compared to the prevalence of 1-2% of self-reported NSAID-related hypersensitivity in a general population [189]. There are few comprehensive data on NSAID hypersensitivity among patients with mastocytosis published to date. One retrospective study described 20 patients who received ASA in varying dosages and schedules. Two patients (10%) reported a mild reaction: either delayed urticaria or immediate flushing [182]. Other descriptive population studies reported a prevalence ranging between 2.3% and 6%, of mostly mild immediate-type reactions [47,48,190]. We could not find published proof of fatal anaphylaxis due to NSAIDs in patients with mastocytosis. Conversely, in a population of 137 persons with drug- or food related anaphylaxis, mastocytosis was found in only 2 patients [191]. Moreover, there was no association between NSAID hypersensitivity and serum tryptase levels in a general cohort [192].

An EAACI position paper advises that patients with mastocytosis and known NSAID tolerance can safely keep taking NSAIDs, but all others should undergo work-up [57]. However, that work-up is not further specified in this paper. Based on our current results, we suggest that everyone with mastocytosis who has never experienced a hypersensitivity reaction to NSAIDs, or other drugs, can safely start taking NSAIDs at home. Given the fact that patients who have experienced a hypersensitivity reaction to another drug appear to be at a higher risk for NSAID hypersensitivity reactions, it seems appropriate to administer the first dose in a clinical setting, preferably with an incremental challenge protocol. As mentioned before, the interpretation of such challenges is very

delicate and requires experience in this area. Despite the placebo-controlled approach, some patients will have only subjective symptoms to an NSAID, and although this is likely to be a 'placebo reaction', it cannot be excluded that these minor symptoms reflect some reaction to the NSAID. The risk of developing more serious reactions in the future is unclear for these patients, and careful counselling is of paramount importance.

Possibly, patients with a history of NSAID-related hypersensitivity reactions can also be challenged with another NSAID. Depending on the type and severity of the previous reaction(s), it can be safer to challenge with a selective COX-2 inhibitor in these cases. Unfortunately, our study cannot corroborate this suggestion. Prospective placebo-controlled studies on these topics would be highly interesting, although are hindered by potential safety issues. On a final note, although the possible benefits of ASA and NSAIDs in general for patients with mastocytosis are clear, the indication must be weighed against possible adverse effects. For instance, patients with mastocytosis already are at risk for peptic ulcer disease [193], which might be increased by the use of NSAIDs. Moreover, it is well-known that NSAIDs can act as a cofactor in anaphylaxis. Ultimately, a careful consideration of risks and benefits needs to be made for each individual, and patients should be consulted on the possible risks.

Conclusions

In summary, the frequency of NSAID hypersensitivity among patients with mastocytosis was 2%, as determined by a prospective double-blind ASA challenge. The frequency of self-reported NSAID hypersensitivity in a retrospective cohort was 4.1%. Based on the mild reactions we saw in our study, combined with the real-life experience that all patients with severe NSAID hypersensitivity experienced these reactions prior to their diagnosis of mastocytosis, we conclude that it is safe to administer NSAIDs to most patients with mastocytosis if they do not have a history of prior NSAID hypersensitivity reactions. Extra caution might be taken in patients with previous hypersensitivity reactions to other drugs, or with traditional risk factors for NSAID hypersensitivity.

Chapter 5

Translational data to improve future treatment options

5.1 The JAK1/JAK2- inhibitor ruxolitinib inhibits mast cell degranulation and cytokine release.

M.A.W. Hermans, B.J. Schrijver, J.C.P.A. van Holten-Neelen, R. Gerth van Wijk, P.M. van Hagen, P.L.A. van Daele, W.A. Dik

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Abstract

Background: Mastocytosis is characterized by the accumulation of aberrant mast cells (MC). Patients suffering from mastocytosis suffer from a wide range of symptoms due to increased levels of MC mediators. It would therefore be of great benefit to inhibit MC mediator release. However, to date there are few drugs available that are known to effectively lower MC mediator levels. The evidence for the involvement of the janus kinase 2 (JAK2) - signal transducer and activation of transcription 5 (STAT5) signaling pathway in MC activation is slowly accumulating. Interference with the JAK2-STAT5 pathway might inhibit MC mediator release. Ruxolitinib, a JAK1/JAK2 inhibitor, indeed decreases symptoms like pruritus and fatigue in patients with myeloproliferative neoplasms. Yet, detailed studies on how ruxolitinib affects human mast cell activity are lacking.

Objective: To investigate the effect of JAK1/2-inhibition with ruxolitinib in the human mast cell lines LAD2 and HMC1.

Methods: LAD2 and HMC1 were stimulated with substance P, codeine or the calcium ionophore A23817. The effect of ruxolitinib on mast cell degranulation (via measurement of β-hexosaminidase, histamine release and CD63 membrane expression) and IL-6, IL-13, MCP-1 and TNF-α production was investigated. The involvement of STAT5 activation was explored by using the selective STAT5 inhibitor pimozide.

Results: Ruxolitinib effectively inhibited codeine- and substance P-induced degranulation in a concentration-dependent manner. Ruxolitinib also significantly inhibited the production of IL-6, TNF-α and MCP-1 as induced by A23817 and substance P. Selective STAT5 inhibition with pimozide resulted in diminished degranulation and inhibition of cytokine production as induced by A23817 and substance P.

Conclusions & clinical relevance: This study demonstrates that the JAK1/JAK2 inhibitor ruxolitinib can inhibit MCactivity, possibly through prevention of STAT5 activation. This renders the JAK-STAT pathway as an interesting target for therapy to release symptom burden in mastocytosis and many other MC mediator-related diseases.

Introduction

Mast cell related diseases such as systemic mastocytosis or mast cell activation syndrome often cause debilitating symptoms mainly due to mast cell (MC)-derived mediators.[91,103,194] Currently, these symptoms are treated symptomatically with histamine antagonists, leukotriene antagonists, cromolyn acid and acetylsalicylic acid.[124] However, this regime is often insufficient to reduce symptoms to an acceptable level in the daily life of patients: especially anaphylaxis, gastrointestinal symptoms or flushing can greatly influence the quality of life.[52]

Most recent studies on the treatment of mastocytosis focus on the application of tyrosine kinase inhibitors (TKI) in the advanced forms of systemic mastocytosis (SM). These inhibitors mainly act through targeting of the KIT-receptor to inhibit MC growth.[75,124,195] Some of these TKI are only effective in MC with wild-type KIT while most patients with mastocytosis harbor the activating D816V KIT mutation.[80] Although newer TKI like midostaurin are also effective in patients with the D816V KIT mutation ,its use is often hampered by gastrointestinal adverse effects.[75] Whereas the search for effective therapies for advanced mastocytosis has been going on for years, it is recognized only recently that TKI might also be of benefit in patients with indolent mastocytosis. The indolent subtype has an excellent prognosis regarding survival, but patients regularly experience symptoms caused by the released MC-mediators rather than from actual tissue invasion by MC. Clinical experience with KIT-targeting TKI in indolent systemic mastocytosis is limited. The multi-TKI masitinib was found to diminish MC mediator-related symptoms such as fatigue and pruritus in 8-25% of patients.[70] Next to well-known mediators such as histamine, leukotrienes and tryptase, MC produce numerous cytokines which are associated with constitutional symptoms in mastocytosis.[7,64,196,197] Therefore, medication that inhibits the release of these MC mediators would be a valuable addition to the, currently limited, therapeutic arsenal for indolent systemic mastocytosis.

Janus kinase (JAK) molecules are involved in the intracellular transduction of signals from multiple receptors, leading to downstream activation of signal transducer and activation of transcription (STAT) molecules and subsequent cellular responses.[198] Of the different JAK- and STAT molecules and signaling pathways currently known, the JAK2-STAT5 pathway is considered the most important for growth and survival of MC.[199] STAT5 also plays a role in IgE-mediated MC degranulation, rendering the JAK2-STAT5 pathway an interesting target for the inhibition of MC activation.[200] In support of this, it has previously been shown that the JAK1/JAK2 inhibitor ruxolitinib attenuates ovalbumin-induced passive systemic anaphylaxis in mice, while another study demonstrated that several JAK2- and STAT5-inhibitors were able to inhibit activation of canine mastocytoma cell lines.[201,202] Furthermore, two recent case studies of patients with systemic mastocytosis reported that MC mediator-related symptoms decreased upon treatment with ruxolitinib.[203,204]

Although these data suggest that JAK2-STAT5 inhibition might effectively reduce the release of MC mediators, basal data on its actual effect on human MC are scarce. The next step towards safe implementation of JAK-STAT inhibitors in the treatment of mastocytosis would be to further explore their exact effects on human MC. To investigate this, we conducted *in vitro* studies with ruxolitinib, using two different human mast cell lines and several parameters of MC activation.

Materials and methods

Cell culture

Two different human mast cell lines were used: HMC1 and LAD2. HMC1 cells grow independently of stem cell factor (SCF) as a result of an activating KIT mutation (kindly provided by dr. Butterfield, Mayo Clinic, Rochester, Minnesota)[205]. DNA sequencing confirmed the presence of the G560V and D816V mutation in KIT. LAD2 is a SCF dependent mast cell line representing wild-type human MC (kindly provided by drs. Kirshenbaum and Metcalfe, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland)[22].

HMC1 cells were cultured in RPMI+ medium (Lonza, Verviers, Belgium) supplemented with 10% fetal calf serum, 50 μM β-mercapto-ethanol (Sigma-Aldrich, St. Louis, Missouri), and antibiotics (penicillin/streptomycin, Lonza). LAD2 cells were cultured in Stem Pro-34 medium supplemented with 2.6% nutrient supplement (both Life Technologies, Grand Island, New York), 2 mM ultraglutamine (Lonza), 100 ng/ml SCF (R&D systems, Abbingdon, U.K.), and antibiotics (penicillin/streptomycin).

Beta-hexosaminidase release

MC degranulation was measured by β-hexosaminidase assay, as adopted from Rådinger et al.[206] In brief, $2x10^4$ LAD2 cells in 100 μL per condition were transferred to a 96-well plate in calcium free phosphate-buffered saline (PBS). The cells were incubated for 30 minutes with varying concentrations of ruxolitinib (Apex Bio, Houston, Texas) or the STAT5-inhibitor pimozide (R&D systems) before further stimulation. Cells were stimulated with 40 μg/ml of codeine (Clinical pharmacy of Erasmus MC, Rotterdam, The Netherlands) or 5 μM of substance P (R&D systems) for 15 minutes at 37°C. Hereafter, plates were centrifuged at 400 G for 4 minutes. Subsequently, 50 μL of supernatant was added to 100 μL of *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide (p-NAG, Sigma-Aldrich) in citrate buffer (pH 4.5), while the cells were lysed by adding 150 μL 0.1% Triton X solution (Sigma-Aldrich). Fifty μL of the cell lysate was added to 100 μL of p-NAG in a different plate. After incubation for 90 minutes at 37°C, the reaction was stopped by adding 100 μL of glycine 400 mM to each well. Optical density (OD) values were measured at 405 and 620 nm using a ELISA plate reader. The relative β-hexosaminidase release was calculated as follows: (2x Δsupernatant)/(Δsupernatant + (4*Δcell lysate)) = % β-hexosaminidase release. Δsupernatant = OD value supernatant – OD value blank condition.

Histamine release

For the determination of histamine release, LAD2 cells were used. The cells were incubated with varying concentrations of ruxolitinib and subsequent-

ly stimulated with codeine for 15 minutes, according to the protocol as described above for the β-hexosaminidase assay. Histamine levels in the supernatant were measured by ELISA (IBL Solutions, Rorsachenberg, Switzerland).

Surface membrane CD63 expression

MC degranulation is accompanied by the movement of CD63 to the external surface of the membrane, and CD63 expression can therefore be used to measure MC degranulation.[207] LAD2 cells were suspended in PBS to a concentration of $3x10^5$ cells in 500 µL per tube. The cells were first incubated with ruxolitinib or pimozide for 30 minutes, and subsequently stimulated with codeine at a concentration of 40 µg/mL for 15 minutes at 37°C. The cells were stained with FITC-conjugated anti-CD63 antibody (ThermoFisher, Waltham, Massachusets). Membrane CD63 expression was analyzed on a flowcytometer (LSRII, Becton Dickinson, Franklin Lakes, New Jersey).

Cytotoxicity assay

Cytotoxicity of ruxolitinib and pimozide was tested by several methods. Firstly, HMC1 and LAD2 cells were cultured for up to 24 hours in the presence of varying concentrations of ruxolitinib (from 50 nM to 50 µM) and pimozide (2 µM to 100 µM). The cells were assessed by microscopy and trypane blue exclusion. Secondly, LDH levels in supernatant of cell cultures of 24 hours were measured by a standard colorimetric assay kit (Roche, Basel, Switzerland).

Cytokine production

For the measurement of cytokine and chemokine production, HMC1 cells were seeded in a 96-well plate at 2 x 10^5 cells in 200 µL culture medium per condition. Subsequently, the cells were incubated for 30 minutes with ruxolitinib (or pimozide) before stimulating them with 1 µM of A23187 or 5 µM of substance P. After testing several time periods of stimulation ranging between 6 and 24 hours for the optimal duration, TNF-α levels in the supernatant were measured after 6 hours of stimulation, and MCP-1 and IL-6 levels were measured after 24 hours of stimulation. See the results section for fur-

ther details. Cytokines were measured by ELISA (all R&D systems, except for IL-6 which was from ThermoFisher). The choice of cytokines was based on previous research on cytokine levels in patients with mastocytosis and other myeloproliferative diseases.[208-210]

Statistical analysis

Graphpad Prism 5 (San Diego, California) was used to analyze most data except for the flow cytometry data, for which FlowJo (Ashland, Oregon) was used. One-way Analysis of Variance (ANOVA) and subsequent Dunnett post-hoc tests were used to determine the statistical significance between the ruxolitinib- or pimozide-treated conditions and the positive control conditions. IC_{50} values were calculated for the inhibitory effects on degranulation and cytokine production of both compounds. The results of β-hexosaminidase, histamine and cytokines/chemokine measurements are depicted in bar graphs as mean values with standard error of the mean (SE). CD63 expression is depicted in histograms as mean fluorescent intensity.

Results

Compound control

Ruxolitinib was not cytotoxic to either HMC1 as LAD2 cells up to a concentration of 50 μM as determined by microscopy, trypane blue exclusion and by LDH release assay for up to 48 hours of incubation. Pimozide was cytotoxic to both cell lines from a concentration of 50 μM and higher as determined by LDH release assay. The vehicle, DMSO, did not affect degranulation up to a concentration of 5%, as measured by β-hexosaminidase release and CD63 expression (data not shown).

Degranulation

First, titration of the stimuli was performed to find the optimal duration and concentration of stimulation for the β-hexosaminidase assay (figure S1) and flow cytometry (figure S2). HMC1 cells appeared unsuitable for these degranulation assays because their degranulation could not be further enhanced by

stimulation with codeine, as measured by β-hexosaminidase release (figure S3). For HMC1 cells, there also was no significant upregulation of CD63 expression upon stimulation with codeine (data not shown). Therefore, only LAD2 cells were used for all degranulation assays. Optimal concentrations to stimulate degranulation were 40 μg/ml for codeine, and 5 μM for substance P, for a duration of 15 minutes.

Ruxolitinib effectively inhibited substance P- and codeine-induced degranulation of LAD2 cells, in a concentration-dependent manner reaching statistical significance at 50 μM and 10 μM, (p 0.028 and p 0.006, respectively; figure 1A and B). This corresponded to an IC_{50} of 7.9 μM (95% CI 2.3-27.3) for substance P-induced degranulation, and an IC_{50} of 10 μM (95% CI; 8.9 – 12.6) for codeine-induced degranulation. Ruxolitinib also inhibited codeine-induced histamine release and CD63 expression of LAD2 cells, however without reaching statistical significance (figure 1C and D, respectively).

Figure 1. The effect of ruxolitinib on degranulation of LAD2 cells.

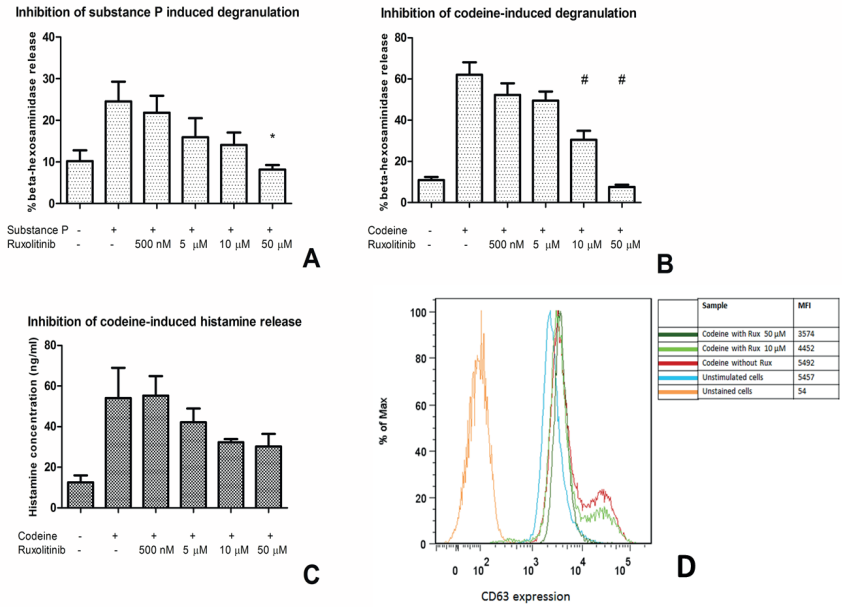
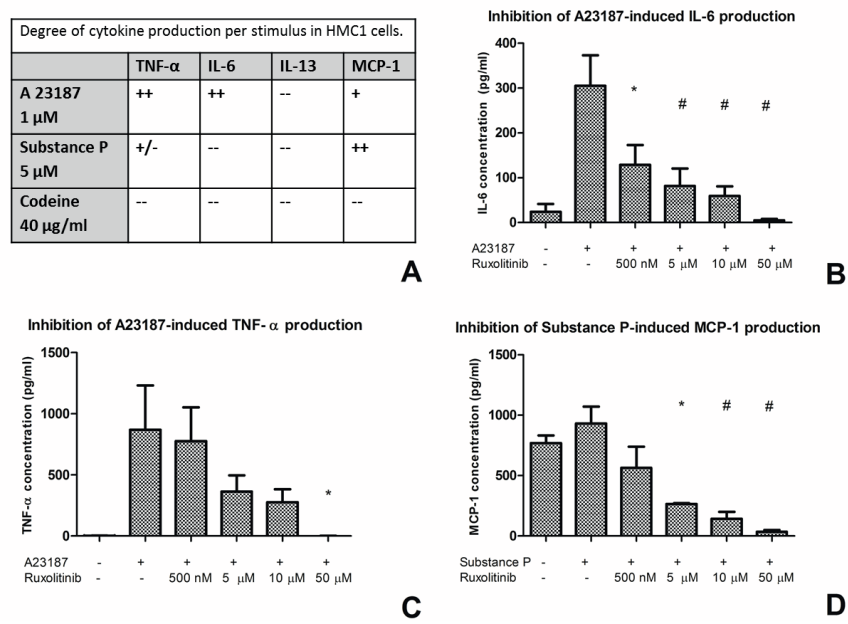


Figure 1. Ruxolitinib effectively inhibits degranulation of LAD2 cells in a dose-dependent manner. **A**: Beta-hexosaminidase assay for substance P induced degranulation. Concentration of Substance P: 5 µM. **B**: Beta-hexosaminidase assay for codeine induced degranulation. Concentration of codeine: 40 µg/ml. **C**: Inhibition of histamine release after stimulation with codeine 40 µg/ml. **D**: Inhibition of CD63 expression after stimulation with codeine 40 µg/ml. MFI= mean fluorescent intensity. *p<0.05, and #p<0.01 as compared with positive control. All bars are depicting the mean of 3 repeated experiments with SE.

Cytokine and chemokine production

HMC1 cells were used for all experiments performed to evaluate cytokine and chemokine production. First, experiments were performed to determine which substance and duration of stimulation was optimal for the induction of IL-6, TNF- α, MCP-1 and IL-13 production in HMC1 cells. This revealed that the optimal stimulation time to induce production of IL-6 and MCP-1 was 24 hours, and that a stimulation time of 6 hours was optimal for TNF-α. This trend was seen regardless of which stimulus was used. A23187 was a more potent stimulus for IL-6 and TNF-α production, whereas Substance P was the best stimulus for MCP-1 production (summarized in figure 2A). Since the IL-13 production was low, regardless of the stimulus used, this was not further pursued. Codeine did not induce the production of detectable levels of any of the tested cytokines.

Figure 2. The effect of ruxolitinib on cytokine and chemokine production by HMC1 cells.



Degree of cytokine production per stimulus in HMC1 cells.				
	TNF-α	IL-6	IL-13	MCP-1
A 23187 1 μM	++	++	--	+
Substance P 5 μM	+/-	--	--	++
Codeine 40 μg/ml	--	--	--	--

A

Figure 2. After establishing the effect of A23187, codeine and substance P on the production of various cytokines (**A**), experiments were performed with the optimal stimulus for each cytokine. Incubation with ruxolitinib lead to decreased production of IL-6 (**B**) and TNF-α (**C**) after stimulation with A23187 for 24 or 6 hours, respectively. The production of MCP-1 after stimulation with substance P for 24 hours was also inhibited (**D**). *p<0.05, and #p<0.01 as compared with positive control.

Ruxolitinib effectively inhibited the production of IL-6, TNF-α, and MCP-1 by HMC1 cells in a concentration-dependent manner (figure 2B-D). For IL-6 production, a statistically significant effect was reached at 500 nM of ruxolitinib and higher (p 0.047 for 500 nM compared with positive control). For TNF-α, the highest level of ruxolitinib (50 μM) displayed significant inhibition (p 0.038). MCP-1 production was significantly inhibited by ruxolitinib 5 μM and higher (p 0.021). The IC_{50} for the inhibition of IL-6 production by ruxolitinib was 7.6 μM (95% CI 6.0 – 9.7), and the IC_{50} for the inhibition of TNF-α production was 1.3 μM (95% CI 0.2 – 9.1). The IC_{50} for MCP-1 production was 4.1 μM, however with a wide 95% CI (0.4 – 43).

Pimozide

To determine the contribution of STAT5 to MC degranulation and cytokine production, we investigated the effect of pimozide, a selective STAT5 inhibitor. Pimozide partly inhibited substance P- and codeine-induced degranulation of LAD2 cells in a concentration-dependent manner, however the obtained decrease in beta-hexosaminidase release was not statistically significant (figure 3). Pimozide had a more potent inhibitory effect on cytokine production by HMC1 cells. The A23187 induced production of IL-6 and TNF- α by HMC1 cells was already significantly reduced at the lowest concentration of pimozide of 5 μM (p 0.002 and p 0.029 respectively, figure 4A & B). Similarly, the substance P-induced MCP-1 production was also inhibited, reaching statistical significance at a concentration of 20 μM (p 0.002; figure 4C). Of note, the spontaneous production of MCP-1 appeared to be relatively high in repeated experiments. The IC_{50} for the inhibition of IL-6 production by pimozide was 7.4 μM (95% CI 3.1 – 17.9), the IC_{50} for TNF-α production by pimozide was 8.0 μM (95% CI 3.7 – 17.1). The IC_{50} for MCP-1 production was 13.9 μM (95% CI 7.1 –27.2).

Figure 3. The effect of pimozide on degranulation of LAD2 cells.

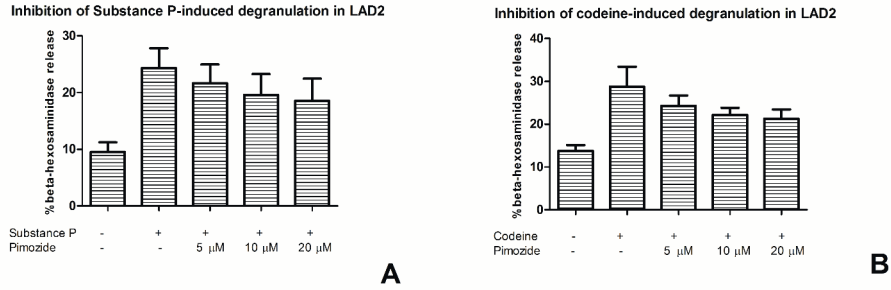
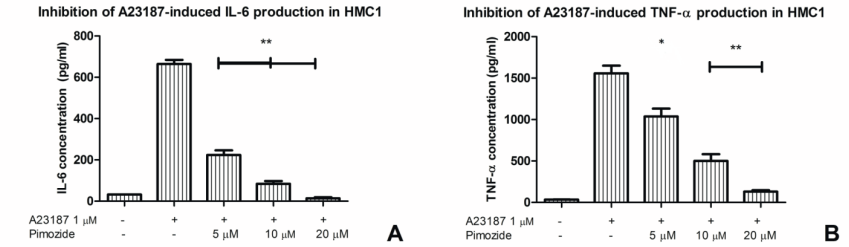


Figure 3. As measured by beta-hexosaminidase release assay, pimozide partly inhibits degranulation of LAD2 in a dose-dependent manner. This effect was similar for stimulation with substance P 5 μM (**A**) and codeine 40 μg/ml (**B**). All bars are depicting the mean of 3 repeated experiments with SE.

Figure 4. The effect of pimozide on cytokine and chemokine production by HMC1 cells.



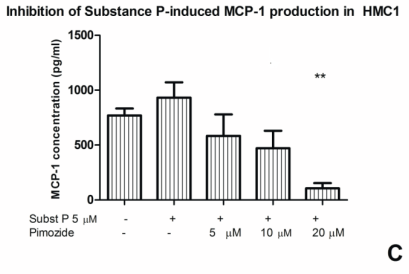


Figure 4. Incubation with pimozide lead to decreased production of IL-6 (**A**) and TNF-α (**B**) after stimulation with A23187 for 24 or 6 hours, respectively. The production of MCP-1 after stimulation with substance P for 24 hours was also inhibited (**C**). *p<0.05, and **p<0.001 as compared with positive control. All bars are depicting the mean of 3 repeated experiments with SE.

Discussion

Here, we provide evidence that the JAK1/JAK2 inhibitor ruxolitinib can efficiently inhibit MC degranulation as well as the production of cytokines. Since substance P and codeine are ligands for G-protein coupled receptors (GPCRs) [13,59], this work implies a link between the JAK2-STAT5 pathway and GPCRs. These stimuli were chosen because it is well-known that codeine can cause MC degranulation, as is confirmed by the fact that many patients with mastocytosis experience MC mediator related symptoms after ingestion of codeine.[211] However, since codeine is an exogenous stimulus, we repeated most experiments with substance P and/or A23187.

In our study, ruxolitinib clearly inhibited MC activity, but its exact intracellular mechanism is not fully elucidated yet. JAK2, and subsequently STAT5, are situated downstream of KIT in MC [200], and thereby involved in the proliferation and survival of MC.[199] The involvement of the JAK2-STAT5 pathway in MC degranulation is less well-described, although there is some evidence available. The role of STAT5 in IgE-mediated MC degranulation has been investigated by several groups, and it is now accepted that STAT5 is activated downstream of FcεR1.[19,200,212] In line with this, previous work has proven that JAK2 is involved in IgE-mediated leukotriene production in mice.[213] Our current data confirm the role of JAK1 and/or JAK2 in MC degranulation and even more so in cytokine and chemokine production. We also found that selective inhibition of STAT5 with pimozide inhibited MC activity, although this did not reach statistical significance for degranulation. Nevertheless, our data support the theory that STAT5 is important for MC activation, although possibly in a lesser extent than JAK2.

Substance P and codeine are ligands to GPCRs. [13,59] Ruxolitinib and pimozide both effectively blocked MC activation as induced by these stimuli. This points at a role for JAK2-STAT5 signaling in GPCR-mediated MC activation. Associations between GPCRs and JAK2-STAT5 signaling have been described in other hematopoietic cell types, for instance for the C-C motif chemokine receptor

5 expressed by T-cells and the platelet activating factor receptor expressed by monocytic cells[214,215] But to our knowledge, our study is the first to suggest an association between GPCRs and a JAK-STAT pathway in MC. The most important signaling pathway downstream of GPCRs involves phosphoinositide 3-kinase (PI3K), which is a versatile tyrosine kinase that can integrate signals from different receptors.[21,216,217] There is some evidence for crosstalk between PI3K and STAT5, specifically in neoplastic MC.[218,219] Possibly, JAK1/JAK2 inhibition by ruxolitinib ultimately interferes with the crosstalk between PI3K and STAT5, although this hypothesis obviously needs further testing.

Ruxolitinib is a rather specific inhibitor of JAK1 and JAK2, yet at high concentrations it is known to inhibit JAK3 as well.[220,221] The IC_{50} of ruxolitinib for JAK3 inhibition was reported to be 438 ± 243 nM in an *in vitro* model for myeloproliferative neoplasms (MPN), which was ~150-200 times higher than the IC_{50} for JAK1 or JAK2 inhibition.[220] The IC_{50} values for ruxolitinib are much higher in MC: they ranged between 1.3 and 10 μM in our study. These values are comparable to a study that investigated the effect of ruxolitinib on murine MC.[201] Since higher concentrations of ruxolitinib are necessary to inhibit MC activation compared with the inhibition of myeloid progrenitor cells, it cannot be formally excluded that the observed effect of ruxolitinib on MC also involves inhibition of JAK3 activity.

Regardless of any (exogenous) stimuli, neoplastic MC constitutively exhibit high levels of phosphorylated STAT5.[18] Additional evidence for the continuous auto-activation of MC in patients with mastocytosis comes from the elevated levels of MC mediators at random measurements in blood and urine of these patients.[15,196,197] The inhibition of the release and/or production of various MC mediators can potentially reduce debilitating symptoms including pruritus, flushing, diarrhea and anaphylaxis, thereby improving the quality of life of these patients. Excessive MC activity also plays a role in many other diseases, including mast cell activation syndrome, chronic spontaneous urticaria, allergies and fibrotic disease. [222,223] The reduction of MC mediator levels

via the inhibition of JAK-STAT signaling might therefore be of therapeutic interest for these diseases as well.

Ruxolitinib is currently only approved for the treatment of the classical myeloproliferative neoplasms (MPN). In patients with MPN, treatment with ruxolitinib leads to a reduction of daily symptoms like abdominal discomfort, pruritus and fatigue.[224,225] In line with our findings, the COMFORT-II trial showed a decrease in the levels of IL6, TNF-α and VEGF in patients with MPN that were treated with ruxolitinib.[226] Given the increased number and activity of MC in bone marrow of patients with MPN it was hypothesized that ruxolitinib exerted its beneficial effect in MPN by inhibition of MC mediator release/production.[227,228] This hypothesis is corroborated by our current study that indeed demonstrates an overall inhibiting effect of ruxolitinib on MC activity. In line with this , two recent case studies showed a convincing decrease of MC mediator related symptoms in systemic mastocytosis upon ruxolitinib treatment [203,204]

Based on our data, combined with other available (pre-)clinical evidence as discussed above, JAK1/JAK2-STAT5 inhibition might represent a promising new therapeutic strategy for patients with mastocytosis and many other MC mediator related diseases. Ultimately, randomized clinical trials are necessary.

Conclusion

We have demonstrated that the JAK1/2 inhibitor ruxolitinib effectively attenuates degranulation and cytokine production by human MC. The fact that pimozide also partly inhibited degranulation, and cytokine production substantially, suggests a role for STAT5 activation in MC activation. JAK2-STAT5 inhibition thus emerges as a new, highly effective, method to lower MC mediator levels. Ruxolitinib, and JAK-STAT inhibition in general, are interesting therapeutic options to reduce debilitating symptoms in mastocytosis and a wide range of other MC mediator-related diseases such as mast cell activation syndrome, chronic spontaneous urticaria, and even fibrotic disease.

Supplementary data belonging to:

The JAK1/JAK2- inhibitor ruxolitinib inhibits mast cell degranulation and cytokine release.

Figure S1. Titration of the optimal concentration of the used stimuli for β-hexosaminidase assay using LAD2 cells.

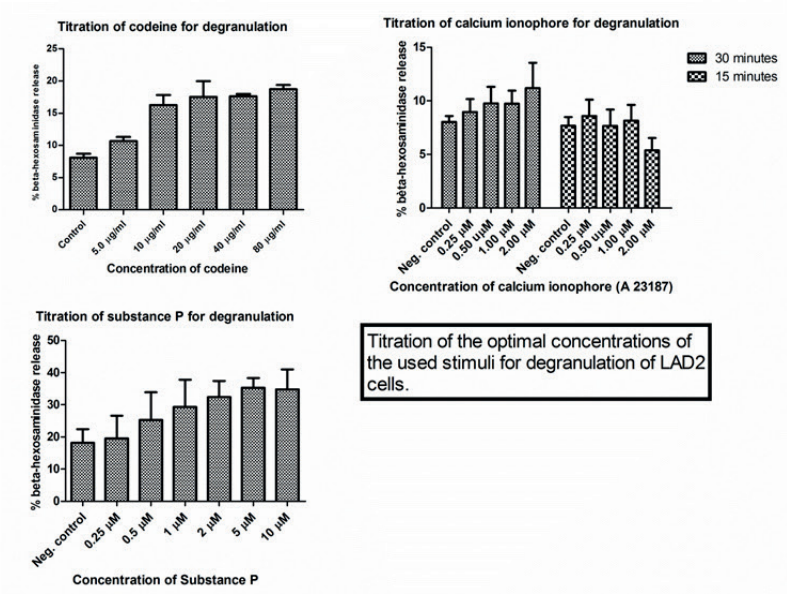
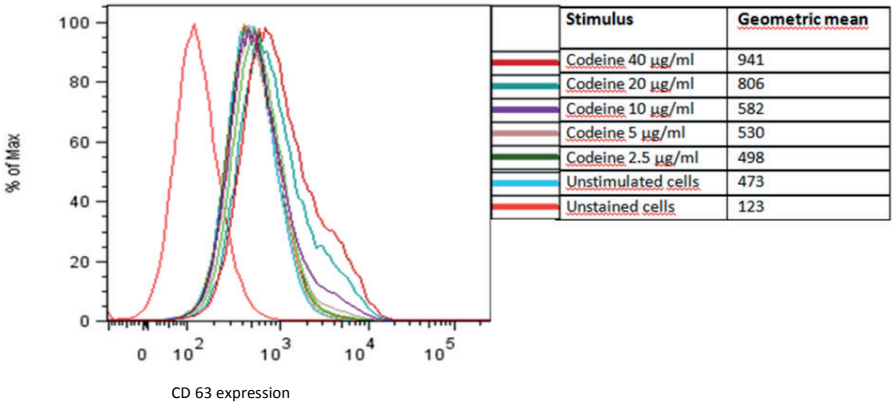
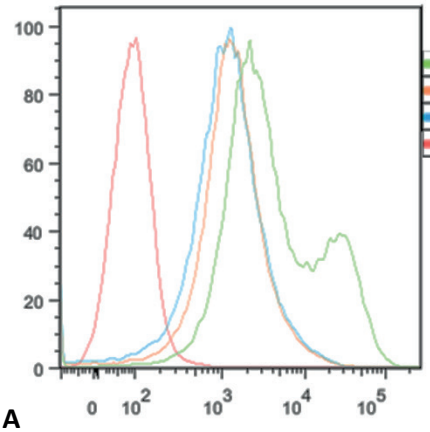


Figure S2. Titration of codeine concentration for detection of CD63 expression by flow cytometry.



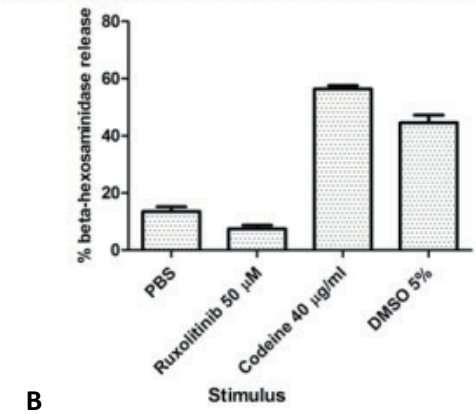
Stimulus	Geometric mean
Codeine 40 µg/ml	941
Codeine 20 µg/ml	806
Codeine 10 µg/ml	582
Codeine 5 µg/ml	530
Codeine 2.5 µg/ml	498
Unstimulated cells	473
Unstained cells	123

Figure S3. Substance controls for measurement of CD63 expression by flow cytometry (A) and for measurement of beta-hexosaminidase assay (B). For the ruxolitinib control, cells were incubated with ruxolitinib 50 mM for 30 minutes, after which the beta-hexosaminidase release was measured as described. For the DMSO control, cells were incubated in PBS supplemented with DMSO 5% and subsequently stimulated with codeine 40 mg/ml for 15 minutes.



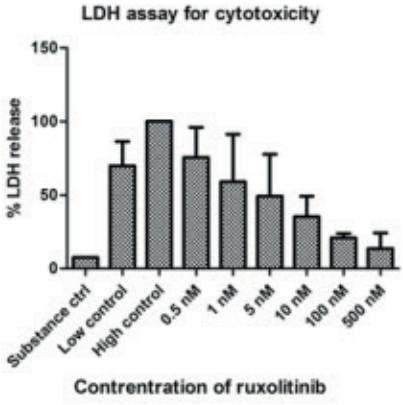
Sample name	Geometric mean
DMSO 5% with codeine 40 µg/ml	3900
Ruxolitinib 50 µM control	1181
Stained control	1026
Unstained control	85.9

A



B

Figure S4. Cytotoxicity of ruxolitinib was ruled out by LDH assay.



Cytotoxicity was excluded by measuring the LDH release by HMC1 cells after incubation with incremental doses of ruxolitinib for 24 hours. Columns depinct medians with IQR.

5.2 Increased group 2 innate lymphoid cells in peripheral blood of adults with mastocytosis.

E.K. van der Ploeg*, M.A.W. Hermans*, V.H.J. van der Velden, W.A. Dik, P.L.A. van Daele, R. Stadhouders. *both authors contributed equally

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Abstract

Background: Systemic mastocytosis is a hematological disease in which aberrant mast cells accumulate due to gain-of-function mutations in the KIT receptor. Group 2 innate lymphoid cells (ILC2s) are effector cells of type 2 immune responses that also express KIT and co-localize with mast cells at barrier tissue sites. In mouse models, mast cell-ILC2 crosstalk can drive local inflammation. However, a possible role for ILC2s in the pathophysiology of mastocytosis remains unexplored. Here, we characterized circulating ILC2s in a clinically diverse cohort of mastocytosis patients.

Methods: We included 21 adults with systemic mastocytosis and 18 healthy controls. Peripheral blood ILC2 abundance and phenotype were analysed by flow cytometry and correlated to clinical characteristics, including the presence of the D816V KIT mutation.

Results: ILC2 levels were significantly higher in D816V$^+$ mastocytosis patients compared to D816V- patients or healthy controls. We observed increased proportions of KIT$^+$ ILC2s among patients with mastocytosis, regardless of D816V status. Patients with skin involvement and itch showed the highest levels of ILC2s, which was independent from atopy or serum tryptase levels. Allele-specific quantitative PCR showed that the vast majority of ILC2s did not carry the D816V mutation.

Discussion: Our findings suggest a role for ILC2s and pathogenic ILC2-mast cell crosstalk in mastocytosis. We hypothesize that a high cutaneous D816V$^+$ mast cell burden alters the skin microenvironment to induce chronic local ILC2 activation and their dissemination into the circulation. Activated ILC2s could contribute to skin symptoms by producing inflammatory mediators and by further augmenting mast cell mediator release.

Introduction

Mastocytosis is a rare myeloproliferative disease in which aberrant mast cells accumulate. Most adult patients have systemic mastocytosis, which is defined by the involvement of at least one extracutaneous organ, most often the bone marrow[46]. In 80-90% of patients with systemic mastocytosis, a somatic D816V mutation is detected in the KIT receptor tyrosine kinase. Under normal conditions, KIT requires binding of its ligand, stem cell factor (SCF), to induce mast cell proliferation and survival[4]. The D816V mutation leads to constitutive ligand-independent activation of KIT signalling, resulting in uncontrolled mast cell proliferation[4]. Patients with systemic mastocytosis can suffer from a wide variety of symptoms, including anaphylaxis, osteoporosis, itching, flushing, dyspepsia, diarrhea, fatigue, and psychological symptoms, which can greatly reduce quality of life[229]. Many of these symptoms are presumably caused by increased levels of mediators released by mast cells such as histamine, tryptase, eicosanoids and pro-inflammatory cytokines. However, it remains unclear why the type and severity of symptoms can vary so greatly between individual patients. In mastocytosis patients, baseline serum tryptase and urine histamine levels do not correlate with individual symptoms[15], suggesting that other cell types might contribute to the pathophysiology of mastocytosis, possibly through their activation by mast cell signals.

Innate lymphoid cells (ILC) orchestrate immune responses at mucosal surfaces in an antigen-independent manner. ILCs can be grouped into various subtypes depending on the inflammatory cytokines they produce, including group 2 ILCs (ILC2s) that play a central role in type 2 immunity and in allergic inflammatory disease[230]. ILC2s and mast cells both reside at barrier sites of the human body, such as the skin, gut and airways. While ILC2s are largely tissue-resident, they can also be detected in the circulation. Interestingly, like mast cells, a subpopulation of ILC2s expresses KIT, and SCF can augment ILC2 activation[231]. In mice, mast cell-ILC2 crosstalk is important to clear helminth infections but also to drive local inflammation[232]. However, whether such crosstalk is also relevant in the context of mastocytosis remains to be addressed.

Methods

Subjects

Adult patients who fulfilled the WHO criteria[33] for indolent systemic mastocytosis were recruited from the Erasmus MC Mastocytosis Center outpatient clinic. Healthy controls were recruited by the Franciscus Gasthuis & Vlietland hospital in Rotterdam. This study was performed according to the latest Helsinki guidelines. All subjects provided a written informed consent and all experimental procedures were approved by the Medical Ethical Committee of the Erasmus MC in Rotterdam, the Netherlands.

Isolation of peripheral blood mononuclear cells and flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation using Ficoll-PaqueTM PLUS (GE Healthcare Life Sciences). ILC2s, ILCPs and CD4+ T cells were analysed using multicolour flow cytometry. See **Supplementary Text** for a detailed description.

Real-time quantitative D816V mutation detection PCR

Genomic DNA was isolated from sorted ILC2 and ILCP fractions. The presence of KIT D816V mutant alleles was evaluated using a previously described233 routine diagnostics real-time quantitative PCR assay. See Supplementary Text for additional details.

Tryptase/IgE measurements and immunophenotyping of neoplastic mast cells in bone marrow

Serum tryptase and IgE levels were measured by fluoroenzymeimmunoassay (FEIA) on a Phadia250 system (Thermo Fisher Scientific) using the ImmunoCAP Tryptase and Total IgE assays according to the manufacturer's instructions. Flow cytometry immunophenotyping of mast cells from bone marrow aspirate is described in the Supplementary Text.

Statistical analysis

Depending on the type of variables, we used nonparametric Mann-Whitney

U tests, chi square tests and Spearman correlation analyses to determine statistical significance. IBM SPSS Statistics (25) and Prism Graphpad software (8.0.2) were used for statistical analyses.

Results and discussion

Twenty-one patients with indolent systemic mastocytosis and 18 healthy controls were included in our study. Sixteen had a detectable D816V KIT mutation in bone marrow samples. Patient characteristics are summarized in Table 1. The percentage of ILC2s was significantly increased in peripheral blood of mastocytosis patients compared to healthy controls, but only in patients carrying the D816V mutation (Fig.1A-B). Although the abundance of circulating KIT^+ ILC precursors (ILCPs) was similar across all groups (Fig.1C), a larger proportion of the circulating ILC2 compartment was KIT^+ in mastocytosis patients, irrespective of D816V status (Fig.1D, Supplementary Fig.1A). Chemokine (CCR4, CCR6) and co-stimulatory (ICOS) receptor expression on ILC2s was similar between groups (Supplementary Fig.1B). $D816V^+$ patients showed significantly higher serum tryptase levels ($p=0.004$) and a trend towards higher neoplastic bone marrow mast cell burden ($p=0.136$) compared to $D816V^-$ patients (Table 1). However, we did not detect a significant correlation between blood ILC2 levels and bone marrow neoplastic mast cell burden (Spearman's $\rho=-0.21$, $p=0.435$) or serum tryptase levels (Spearman's $\rho=-0.073$, $p=0.752$) (Supplementary Fig.2A-B).

While total $CD4^+$ T cell levels did not differ, proportions of $CRTH2^+$ T helper-2 (Th2) cells, the functional counterparts of ILC2s belonging to the adaptive immune system[230], were also significantly increased in peripheral blood of mastocytosis patients compared to healthy controls (Fig.1E, Supplementary Fig.3A-B). However, we found no significant correlation between the levels of $CRTH2^+$ Th2 cells and ILC2s (Spearman's $\rho=0.13$, $p=0.58$).

We next explored associations between circulating ILC2 populations and clinical symptoms in mastocytosis patients. Patients with maculopapular cu-

taneous mastocytosis (MPCM) showed elevated levels of ILC2s as compared to healthy controls and patients without MPCM (Fig.2A). This is in line with the high D816V prevalence (88.2%) in the $MPCM^+$ group and only a single $D816V^+$ patient without MPCM. Additional analysis using clinical data from our complete cohort of 263 adults with mastocytosis confirmed increased skin involvement in $D816V^+$ patients as compared to $D816V^-$ patients: 73% versus 42%, respectively (p=0.002, chi-square test). Compared to healthy controls, $D816V^+$ patients who reported itch had higher levels of ILC2s, irrespective of their KIT expression status (Fig.2B, Supplementary Fig.4A-B).

Table 1. Baseline characteristics of patients and controls.

	Healthy controls (N=18)	Mastocytosis patients (N=21)	D816V+ patients (N=16)	D816V- Patients (N=5)	P-value (D816V+ vs D816V-)
Sex (male/female)	7/11	10/11	7/9	3/2	ns
Age (years)	37.6 (10.4)*[#]	53.0 (15.4)	55.2 (13.8)	46 (5.3)	ns
Serum tryptase (µg/L)	N.A.	51.3 (72.2)	62.9 (78.7)	11.8 (34.6)	0.004
Neoplastic MC burden BM (%) [¶]	N.A.	0.12 (0.13)	0.14 (0.10)	0.04 (0.03)	ns
Total IgE (U/ml)	N.A.	26 (31)	29 (33)	13 (15)	ns
Maculopapular cutaneous mastocytosis (%)	N.A.	81.0%	93.8%	40%	0.008
Itch (%)	N.A.	66.6%	68.8%	60%	ns
Atopy (%) **	33.3%	19.0%	31.25%	0%	ns
Anaphylaxis (%)	N.A.	23.8%	25%	20%	ns
Flushing (%)	N.A.	42.9%	43.8%	40%	ns
Osteoporosis (%)	N.A.	42.9%	50%	20%	ns
Diarrhoea (%)	N.A.	38.1%	43.8%	20%	ns

N.A., not applicable ; ns, not significant ; MC, mast cell ; BM, bone marrow.

* Continuous variables shown as: mean (standard deviation)

** Atopy in mastocytosis patients was defined by clinical symptoms of asthma, rhinoconjunctivitis, and/or proven sensitization to pollen or house dust mite by skin prick test or specific IgE measurement. For healthy controls, atopy was defined as a history of allergic rhinoconjunctivitis and/or food allergy.

[#] Controls were significantly younger than mastocytosis patients (p=0.001)

[¶] Neoplastic mast cell burden in the bone marrow was defined by aberrant expression of CD2 and/or CD25 as measured by flow cytometry.

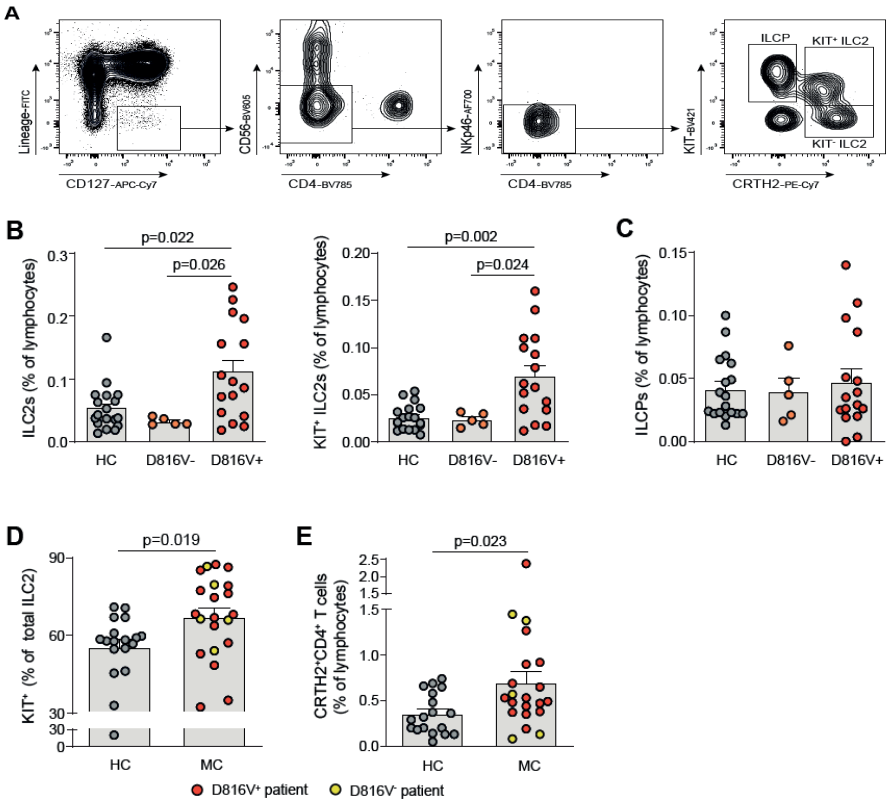


Figure 1. ILC2s are increased in peripheral blood of D816V+ mastocytosis patients. (A) Flow cytometry gating strategy for ILC precursors (ILCP), KIT$^+$ ILC2s and KIT$^-$ ILC2s. **(B)** Proportions of total ILC2s (left) and of KIT$^+$ ILC2s (right) in peripheral blood of healthy controls (HC), D816V negative (D816V-) and D816V positive (D816V+) mastocytosis patients. **(C)** Proportions of ILCPs in peripheral blood of HC, D816V- and D816V+ patients. **(D)** KIT$^+$ ILC2 proportions of total ILC2s in peripheral blood of HC and mastocytosis (MC) patients. D816V- patients are indicated by yellow symbols; D816V+ patients by red symbols. **(E)** Proportions of CRTH2$^+$CD4$^+$ T cells in peripheral blood of HC and MC patients. D816V- patients are indicated by yellow symbols; D816V+ patients by red symbols. Data are shown as symbols for individual patients/controls, together with bar graphs for mean values +SEM. Comparisons between groups were evaluated using a Mann-Whitney U test; $p<0.05$ was considered statistically significant.

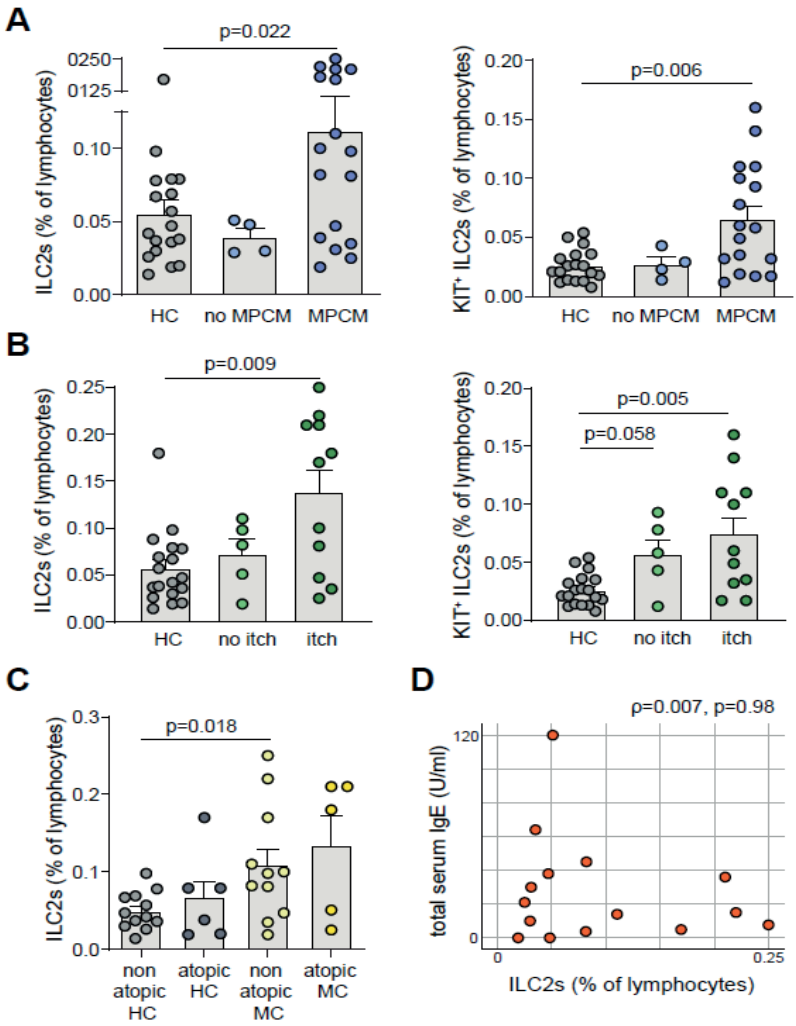


Figure 2. ILC2 levels and phenotype correlate with skin symptoms and atopy. (A) Total (left) and KIT$^+$ (right) ILC2 proportions in all mastocytosis patients with or without maculopapular cutaneous mastocytosis (MPCM). **(B)** Total (left) and KIT$^+$ (right) ILC2 proportions in D816V$^+$ mastocytosis patients with or without itch. **(C)** Total ILC2 proportions in D816V$^+$ mastocytosis patients (MC) and healthy control (HC) subjects with or without atopy. (**D**) Scatter plot showing an absence of correlation between total IgE levels (U/mL) and ILC2 abundance (Spearman's ρ=0.007, p=0.98). Data are shown as symbols for individual patients/controls, with bar graphs in panels A-C showing mean values +SEM. Comparisons between groups were evaluated using a Mann-Whitney U test; $p<0.05$ was considered statistically significant.

Of note, D816V$^+$ patients with or without atopy showed similar levels of ILC2s and we found no significant correlation between serum IgE levels and ILC2 abundance (Spearman's ρ=0.007, p=0.98) (Fig.2C-D). Furthermore, ILC2 abundance and KIT expression were not associated with anaphylaxis, diarrhoea, osteoporosis or flushing, and we found no significant correlations with D816V allelic burden in peripheral blood (p>0.05). CRTH2$^+$ Th2 cell levels were not specifically increased in patients with the D816V mutation, MPCM or itch (p>0.05), emphasizing the specificity of the association between ILC2s and D816V$^+$ mastocytosis with skin involvement.

To assess whether the altered ILC2 compartment in mastocytosis patients is linked to the occurrence of the D816V KIT mutation in the ILC lineage, we performed allele-specific quantitative PCR on isolated genomic DNA from ILC2s and ILCPs. Despite a ~1-2% mutant allele detection sensitivity (see Supplementary Text), we could not detect D816V allelic DNA in ILC2s or ILCPs (Supplementary Fig.5A-B), indicating that the D816V mutation is absent or very rare in circulating ILCs.

Here we describe, to the best of our knowledge, the first explorative investigation into the abundance and phenotype of ILC2s in patients with mastocytosis. We found that patients with the activating D816V KIT mutation in mast cells showed elevated frequencies of circulating ILC2s compared to healthy controls or D816V$^-$ patients. The increased circulating ILC2 levels in D816V$^+$ patients were linked to the presence of MPCM and itch. Together, our observations reveal positive associations between ILC2 frequencies, the D816V mutation and specifically skin symptoms.

Since we could not detect the D816V mutation in peripheral blood ILCs, the increased ILC2 abundance in the circulation of mastocytosis patients with cutaneous symptoms is likely a consequence of excessive mast cell activity in the bone marrow or skin rather than a cell-intrinsic ILC2 defect. However, increased bone marrow output of ILC2s appears an unlikely scenario, as

circulating ILC2 levels did not correlate with bone marrow mast cell burden or serum tryptase levels. Hence, we postulate that constitutive mediator release by D816V$^+$ skin mast cells, including inflammatory molecules such as IL-1β, TGFβand IL-33-activating proteases (e.g. chymase and tryptase)[8], could activate skin-resident ILC2s and create a favourable microenvironment for recruiting circulating ILC2s (Fig.3). Moreover, mast cell-derived prostaglandin D2 has been previously shown to stimulate ILC2 migration via CRTH2 [234]. Furthermore, soluble SCF levels are higher in lesional skin of mastocytosis patients[235], potentially increasing the local SCF availability for KIT$^+$ ILC2s. Chronic ILC2 activation could in turn contribute to a cutaneous cytokine milieu that promotes inflammation, mast cell mediator release and skin symptoms - similar to mechanisms suggested for atopic dermatitis[31]. Prolonged stimulation of tissue ILC2s can trigger their systemic dissemination[236], which can explain the increased ILC2 presence in the circulation in D816V$^+$ mastocytosis. Interestingly, KIT$^+$ ILC2s express the skin-homing chemokine receptor CCR10[231,237], implying that this ILC2 subset is capable of efficiently migrating towards the skin (Fig.3). In line with enhanced skin migration of KIT$^+$ ILC2s in mastocytosis, we observed that patients with frequent itch showed a reduced relative proportion of KIT$^+$ cells within their circulating ILC2 compartment compared to patients without itch.

In summary, we conclude that circulating ILC2s are elevated in D816V$^+$ mastocytosis and are associated with the presence of MPCM and itch, providing a strong rationale for performing in-depth studies into the role of ILC2s in the pathophysiology of mastocytosis.

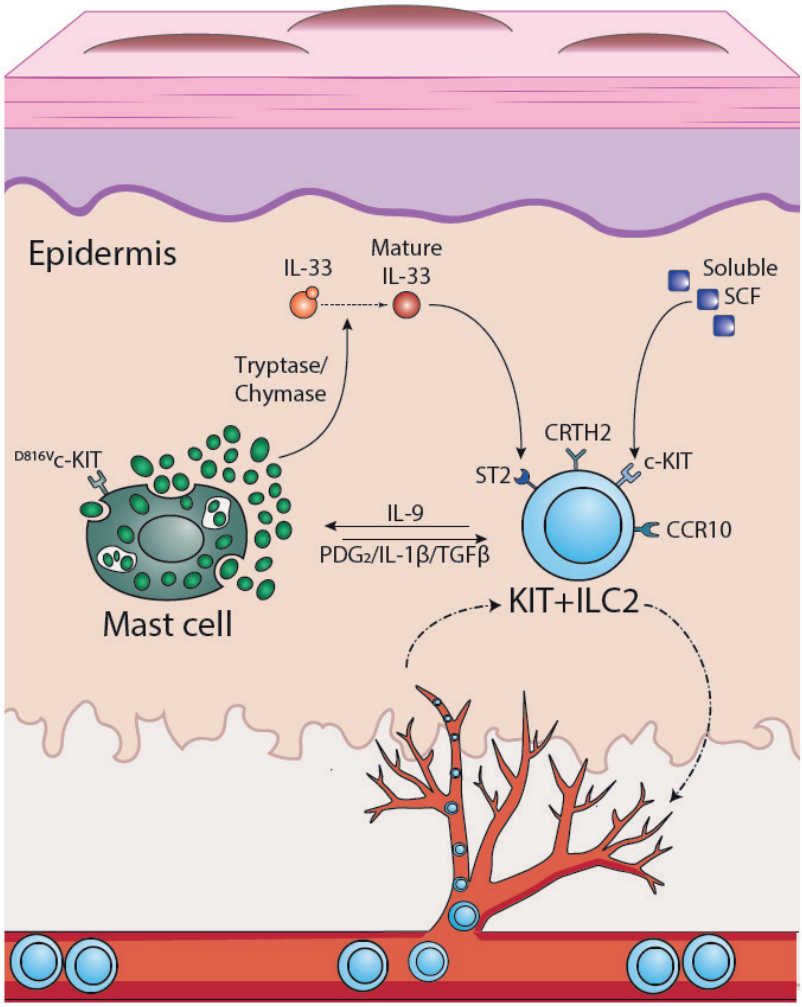


Figure 3. Proposed model for pathological ILC2-mast cell interplay in the skin of D816V⁺ mastocytosis. Constitutive mediator release by D816V⁺ skin mast cells, including inflammatory molecules such as IL-1β, TGFβ and IL-33-activating proteases (e.g. chymase and tryptase) activate skin-resident ILC2s and create a favourable microenvironment for recruiting circulating ILC2s (i.e. via CCR10). Elevated soluble SCF levels further augment KIT⁺ ILC2 activation. Chronic ILC2 activation in turn contributes to an inflammatory cutaneous cytokine milieu (e.g. via production of IL-9) that further promotes mast cell activity and skin symptoms but also triggers ILC2 dissemination into the circulation. Solid arrows denote activation via indicated signaling molecules; dashed arrow indicate cleavage of IL-33 into mature active IL-33; striped arrows depict cellular migration.

Supplementary data belonging to: Increased group 2 innate lymphoid cells in peripheral blood of adults with mastocytosis.

Methods

Flowcytometry analysis and fluorescence assisted cell sorting

PBMCs were subjected to extracellular staining with antibodies for 60 minutes at 4°C, and for 15 minutes at 4°C with LIVE/DEAD™ Fixable Green Dead Cell Stain Kit (Thermofisher). Stainings were performed with five to ten million PBMCs in order to obtain sufficient number of ILCs. The following antibodies to human proteins were used (including manufacturer, dilutions used and clone numbers). From BioLegend: Peridinin chlorophyll protein–cyanine 5.5 (PerCP-Cy5.5)-conjugated anti-CCR4 (1:40, L291H4), Briliant violet (BV)421-conjugated anti-CD117 (1:20, 104D2), Fluorescein isothiocyanate (FITC)-conjugated anti-CD94 (1:20, DX22), Alexa Fluor (AF)700-conjugated anti-NKp46 (1:20,9E2). From Thermofischer: Allophycocyanin (APC)-conjugated anti-CD127 (1:20, eBioRDR5), FITC-conjugated anti-CD3 (1:100, UCHT1), anti-CD14 (1:200, 61D3), PE-indotricarbocyanine (Cy)7-conjugated Streptavidin (1:1000). From Miltenyi Biotec: Biotinylated anti-CRTH2 (1:50, BM16). From Beckman Dickinson: FITC-conjugated anti-CD19 (1:300, HIB19), anti-CD16 (1:400, 3G8), anti-TCRgd (1:20, B1), Phycoerythrin (PE)-conjugated anti-CCR6 (1:20, 11A9), BV650-conjugated anti-ICOS (1:20, DX29), BV605-conjugated anti-CD56 (1:50, NCAM16.2), BV785-conjugated anti-CD4 (1:30, SK3). For flowcytometry analysis, data were acquired on an LSR II or a FACSymphony flow cytometer using FACS Diva software 6.1 (BD Biosciences) and analyzed using FlowJo 10 (BD Biosciences). We sorted ~2000 ILC2s (Lineage-CD127+CRTH2+) or ILCPs (Lineage-CD127+CD117+CRTH2-) in 1.5 ml eppendorf tubes rinsed with 10% fetal calf serum for real-time quantitative D816V mutation detection PCR using a FACSAria (BD Biosciences).

Real-time quantitative D816V mutation detection PCR

Genomic DNA was isolated from sorted ILC2 and ILCP fractions (with a FACSAria, see above) using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. The presence of KIT D816V mutant alleles was evaluated using a previously described[233] (Electronic routine diagnostics real-time quantitative PCR assay that uses wild-type and mutation-specific primer/probe sets. The D816V mutation was deemed undetectable if cycle threshold (CT) values were >38. DNA from HMC1.2 cells were used as a control for the D816V mutant allele and DNA from peripheral blood buffy coats as control for the wild type allele. The quantitative range of the assay was controlled for by including a standardized serial dilution of HMC1.2 or peripheral blood buffy coat genomic DNA in the same run. Accurate detection of both alleles was observed down to the 0.01% dilution (as indicated by the positive control bars in **Supplementary Fig.5**).

Tryptase and IgE measurements

Serum tryptase was measured by FEIA technology using the Phadia250 system (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. Total serum IgE (U/mL) was also measured by fluoroenzymeimmunoassay (FEIA) on the Phadia 250 system using the ImmunoCAP™ Total IgE test (Thermo Fisher Scientific, Uppsala, Sweden), according to manufacturer's instructions.

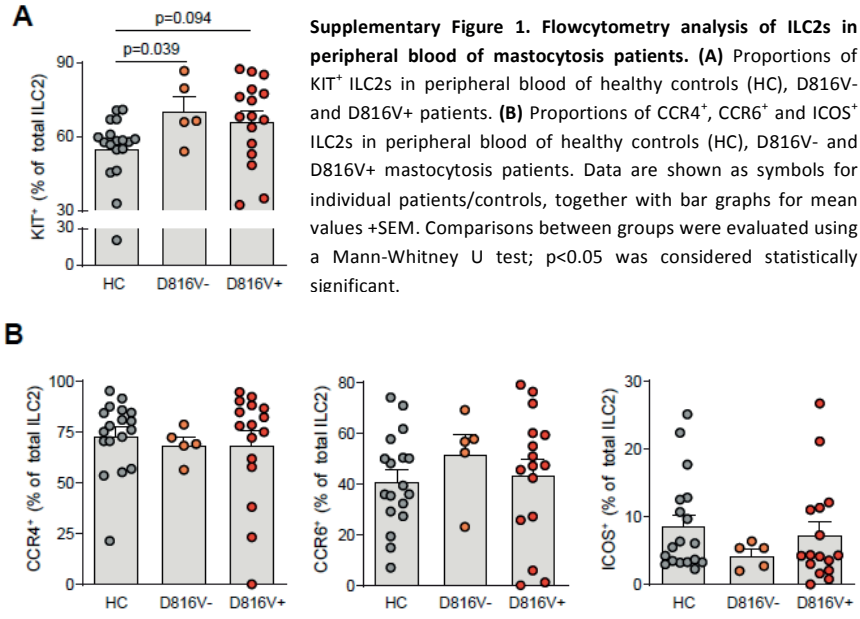
Immunophenotyping of neoplastic mast cells in bone marrow

Flow cytometry immunophenotyping of mast cells from bone marrow aspirate is performed routinely in our lab for diagnostic purposes, and precise methods have been previously described in great detail [238]. Briefly, bone marrow aspirate is collected in heparin tubes and processed within 24 hours. Erythrocytes are lysed using ammonium chloride after which leucocytes are washed with PBS/BSA and resuspended at 6x10^7 cells/mL. Fifty µL of this suspension is stained (10 minutes at room temperature) using the following antibodies: CD117-PE (104D2), CD45-PerCP (2D1), CD25-APC (2A3), CD117-PE-Cy7 (104D2; custom conjugated), CD34-APC-Cy7 (8G12; custom conjugated) (all from BD Biosciences); CD2-FITC (T11), CD2-PE (T11), CD33-PE (My9) (all from Beckman Coulter); and CD117-APC (104D2; Dako Cytomation, Glostrup, Denmark). Subsequently, cells are washed with PBS/BSA and resuspended in FACSFlow Solution (BD Biosciences). Data are acquired using a FACSCalibur or FACS Canto B. Of note, 1x10^6 cells are recorded per tube. Data are analyzed using FACS Diva software. Mast cells are gated based on strong CD117 expression and subsequent gating is performed in the six-colour analysis using CD33 positivity and CD34 negativity. Neoplastic mast cells are CD2 and/or CD25 positive.

Acknowledgements

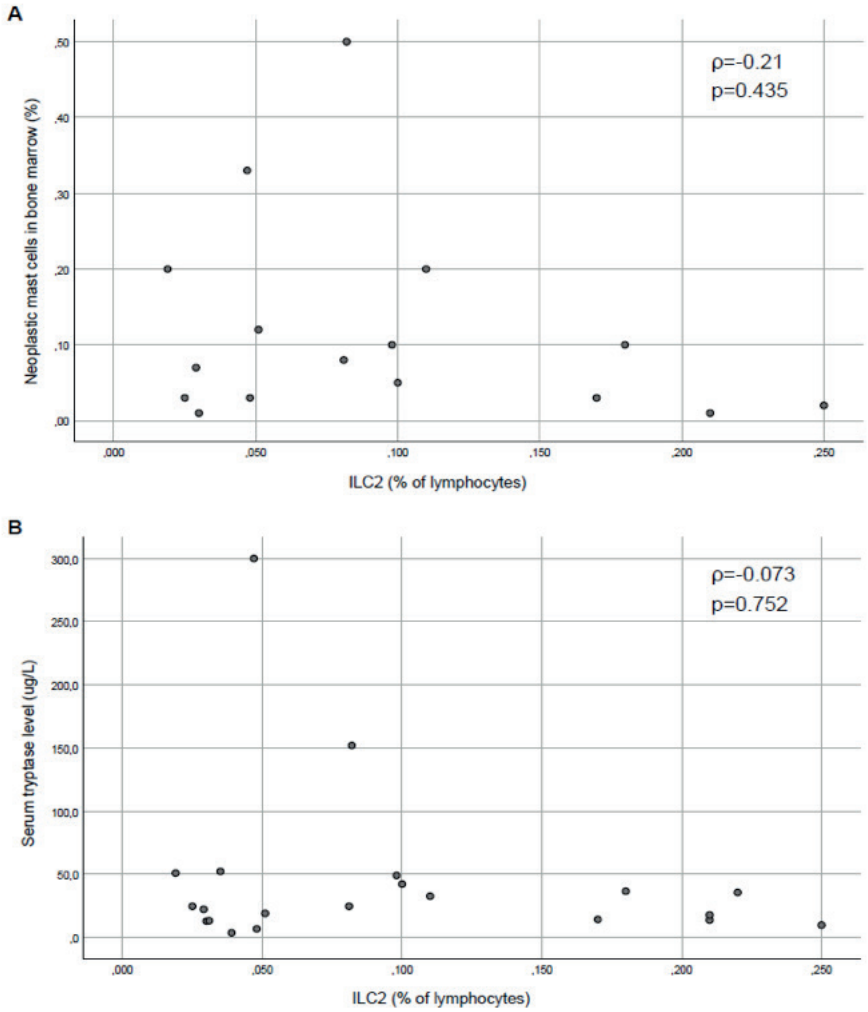
We would like to thank R.W. Hendriks, P.M. van Hagen and R. Gerth van Wijk (Erasmus MC, Rotterdam) for insightful discussions and M. van Nimwegen and M.J.W. de Bruijn (Erasmus MC, Rotterdam) for their technical assistance.

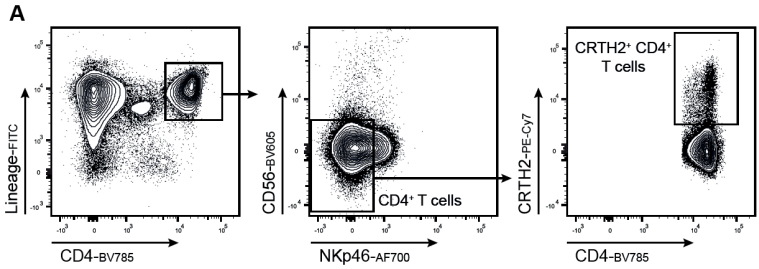
We thank G.M. de Boer, G.J. Braunstahl and G.A. Tramper (Franciscus Gasthuis & Vlietland Hospital, Rotterdam) for inclusion of healthy control individuals.

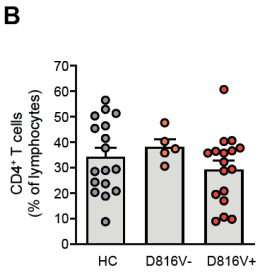


Supplementary Figure 1. Flowcytometry analysis of ILC2s in peripheral blood of mastocytosis patients. (A) Proportions of KIT^+ ILC2s in peripheral blood of healthy controls (HC), D816V- and D816V+ patients. **(B)** Proportions of $CCR4^+$, $CCR6^+$ and $ICOS^+$ ILC2s in peripheral blood of healthy controls (HC), D816V- and D816V+ mastocytosis patients. Data are shown as symbols for individual patients/controls, together with bar graphs for mean values +SEM. Comparisons between groups were evaluated using a Mann-Whitney U test; $p<0.05$ was considered statistically significant.

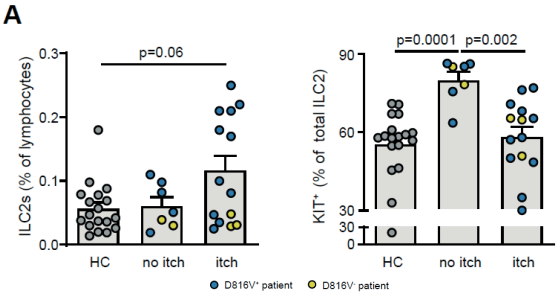
Supplementary Figure 2. Peripheral blood ILC2s levels do not correlate with bone marrow mast cell burden or serum tryptase levels. Scatter plots showing an absence of correlation between ILC2 abundance and neoplastic bone marrow mast cell burden (panel A; spearman ρ=0.21, p=0.435) or serum tryptase levels (panel B; spearman ρ=-0.073, p=0.752). Data are shown as symbols for individual patients/controls.

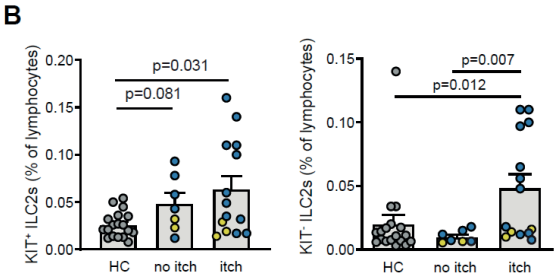






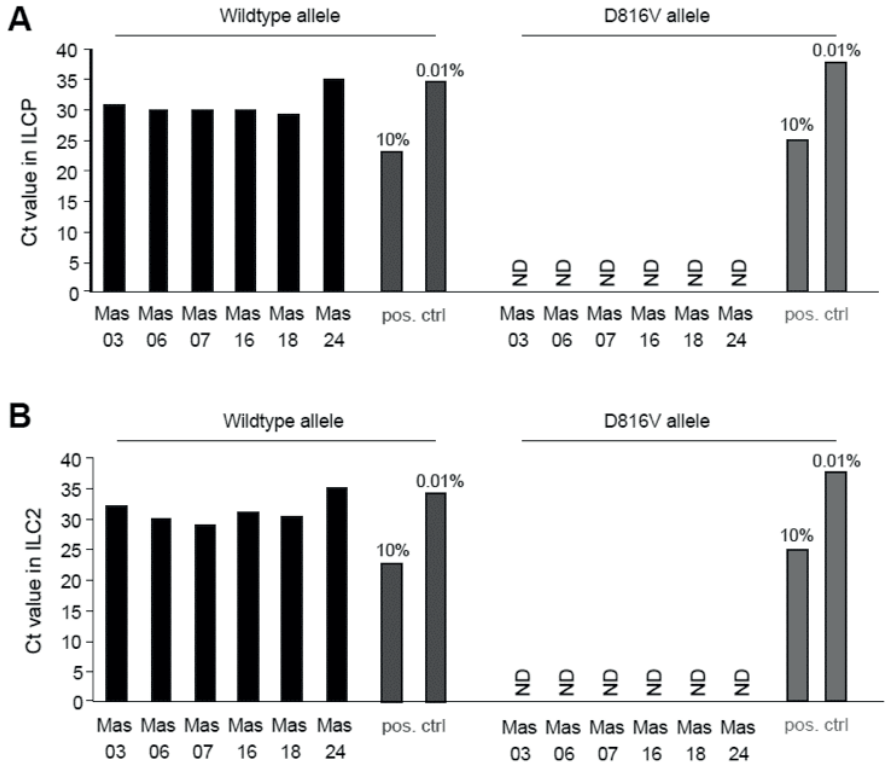
Supplementary Figure 3. Flowcytometry analysis of CD4$^+$ T cells in peripheral blood of mastocytosis patients. (A) Flow cytometry gating strategy for total CD4$^+$ and CRTH2$^+$CD4$^+$ T cells. **(B)** Proportions of CD4$^+$ T cells in peripheral blood of healthy controls (HC), D816V- and D816V+ mastocytosis patients. Data are shown as symbols for individual patients/controls, together with bar graphs for mean values +SEM. Comparisons between groups were evaluated using a Mann-Whitney U test; p<0.05 was considered statistically significant.





Supplementary Figure 4. Associations between itch and circulating ILC2 levels in mastocytosis patients. (A) Total (left) and KIT$^+$ (right) ILC2 proportions in healthy controls (HC) all mastocytosis patients with or without frequent itch. **(B)** KIT$^+$ and KIT$^-$ ILC2 levels in HC and all mastocytosis patients with or without frequent itch. D816V mutation status is indicated for each individual patient (blue: D816V$^+$, yellow: D816V$^-$). Data are shown as symbols for individual patients/controls, together with bar graphs for mean values +SEM. Comparisons between groups were evaluated using a Mann-Whitney U test; p<0.05 was considered statistically significant.

Supplementary Figure 5. Failure to detect D816V allelic DNA in genomic DNA isolated from circulating ILC2s or ILCPs. Shown are cycle threshold (Ct) values from diagnostic quantitative PCR assays for the wildtype and D816V KIT alleles on genomic DNA isolated from ~2000 sorted ILCPs (A) and ILC2s (B) of six mastocytosis patients with detectable D816V KIT alleles in bone marrow samples. Serially diluted DNA from HMC1.2 cells was used as a positive control for the D816V mutant allele; DNA from peripheral blood buffy coats was used as control for the wild type allele. Internal controls ('pos. ctrl') for both PCR assays (10% and 0.01% dilutions) are indicated in the figure. The D816V mutation was deemed undetectable ('ND') if Ct values were >38. Combining the number of input cells, the succesfull amplification of the 0.01% dilution control and the ~8 Ct remaining detection range, this experimental setup translates to a ~1%-2% mutant allele detection sensitivity.



Chapter 6

Discussion and future perspectives

6.1 Discussion and future perspectives

Mastocytosis is an elusive disease that has many faces. Consequently, developments and progress in all aspects of clinical care are evolving rather slowly. In almost every introduction of a paper about mastocytosis, it is stated that it is a rare disease. The prevalence of indolent systemic mastocytosis (ISM) was estimated to be 13:100,000 in The Netherlands in 2013 which is actually not that low [38]. Although different definitions are used internationally, the official definition of a rare disease in the European Union is a prevalence of ≤ 5:10,000 [239]. Of note, the estimated prevalence of 13:100,000 only encompasses ISM and the total prevalence of all subtypes of mastocytosis among all ages must be higher. Although still a rare disease, its rarity cannot be used as an reason to base the clinical care largely on expert opinion. In 2019, 52 publications have been added to the Pubmed database that listed the major Mesh term 'mastocytosis', were written in English and involved human subjects. Only fifteen of these (28.8%) were original studies, the rest were case reports, non-systematic reviews or expert opinion papers. In this thesis, we have tried to find scientific grounds for certain aspects of the common care for mastocytosis. Still, many uncertainties concerning the pathophysiology as well as clinical aspects remain.

Pathophysiological considerations: more than a mast cell disease

Mastocytosis is characterized by an increased amount of phenotypically aberrant mast cells. It seems logical to attribute most symptoms and complications of this disease to the consequences of increased mast cell mediators, and mast cell invasion of organs. However, mast cells are in constant interaction with their direct environment [3,26]. It could therefore by expected that other parts of the immune system and/or adjacent non-immune cells are influenced in case of mastocytosis. Since ILC2s are important in type 2 immunity, are present in the same sites as mast cells, and can express KIT in their mature form [230,231], we considered this an interesting cell type to investigate in mastocytosis. We indeed found that the relative number of ILC2s in peripheral blood was increased in those patients with ISM that harbor the D816V

mutation in KIT (Chapter 5). Since our study was a first exploratory attempt, we could merely hypothesize on the pathophysiological mechanisms behind the demonstrated increase in ILC2s. Given the association of ILC2 abundance with skin involvement and D816V mutation, it appears that the neoplastic mast cell population in the skin induces changes in ILC2 homeostasis. Mast cells can produce many substances that activate ILC2s and have been demonstrated to be essential to enable ILC2 activation in helminth infection [232]. Vice versa, ILC2 activation leads to skin inflammation, providing activating signals to mast cells [31]. However, the increase in ILC2s is more likely to be a result of changes due to mastocytosis than a cause of mastocytosis-related symptoms, since the number of ILC2s did not correlate with other symptoms than skin involvement in our study.

A remarkable additional finding in the study in chapter 5.2 was the significant association between the D816V mutation and skin involvement in patients with SM: 93.8% of D816V+ patients had MPCM versus 40% of D816V- patients. This association has not been published before as a specific finding but might provide insight in the pathophysiology of mastocytosis. Hypothetically, D816V+ mast cells are more likely to migrate to the skin than D816V- mast cells. Another explanation might lie in the fact that D816V+ mast cells do not need SCF to survive, leading to increased availability of SCF to other dermal cells. SCF is naturally present in the skin, where it is produced by keratinocytes and fibroblasts [240]. It was already demonstrated in the 90's that the levels of cell-free SCF are increased in the skin of patients with mastocytosis [241]. Consequently, the proliferation and/or activation of dermal cells that express KIT could be enhanced in mastocytosis, altogether leading to a clinical picture of MPCM. Next to ILC2s, melanocytes also express KIT [242]. In one study in mice, increased availability of keratinocyte-derived SCF led to epidermal melanocytosis [243]. Thereby, melanocytes might be responsible for the hyperpigmentation that is seen in MPCM [244]. Interestingly, the prevalence of malignant melanoma appears to be increased among patients with systemic mastocytosis. One Danish study in fact found a hazard ratio of 7.5 compared with

the general population [179]. The demonstrated increase in both KIT-bearing cell types ILC2s and melanocytes suggests that the altered skin environment in (D816V+) mastocytosis has functional consequences for various other cell types than only mast cells. These, so far hypothetical, mechanisms provide a rationale for research of more cell types than only mast cells in mastocytosis.

Diagnosis

As with every rare disease, an important issue is the lack of awareness among physicians who do not encounter patients with mastocytosis regularly. This led to a significant delay in diagnosis of over 8 years when calculated in 2015 (Chapter 2) [102,103]. Unfortunately, the mean time to diagnosis from the 63 patients in our database who have been diagnosed with mastocytosis between January 1st 2016 and July 1st 2020 is still 9.2 years (standard deviation 9.2). This underlines the task still ahead of us to better inform patients and physicians about mastocytosis. Due to the delay in diagnosis, patients suffer from debilitating symptoms which are potentially avoidable, and they are refrained from preventive measures against anaphylaxis and osteoporosis.

The diagnostic work-up also requires a certain level of expertise. Serum tryptase levels are often used as a screening tool. Yet we have demonstrated in this thesis that serum tryptase levels are not sufficiently reliable to rule out systemic mastocytosis in all adults (Chapter 3.1) [245]. This can be confusing since the WHO set a cut-off for the serum tryptase level of 20 µg/L as a minor criterion for the diagnosis of SM [33]. This cut-off value is seemingly arbitrary, and was never supported by published research. Another paper on this topic also questioned the reliability of a cut-off level of 20 µg/L, although they did not find any systemic mastocytosis in patients with a serum tryptase level <10 µg/L [15]. However, this might represent inclusion bias since this was a retrospective analysis as well. Removal of the minor criterion of a serum tryptase level >20 µg/L could probably increase the detection SM. A prospective study would be necessary to define a more reliable cut-off value for bone marrow biopsy, although other parameters such as mastocytosis in the skin, anaphylaxis and osteoporosis should be taken into account in the diagnostic process.

The development of a sensitive RQ-PCR for D816V mutated KIT in peripheral blood has helped to screen more effectively for SM, especially in patients with Hymenoptera related anaphylaxis, although this assay does not reach a sensitivity of 100% [246]. The exact indications for bone marrow examination for SM remain a topic of discussion. Several algorithms and scoring systems have been developed over time to aid physicians in this clinical decision, but these algorithms were never validated by other researchers than the groups that developed the tools [90,247]. Furthermore, the assessment of the material acquired by bone marrow biopsy requires special expertise. This was demonstrated by the mastocytosis expertise network in Spain: The researchers performed a new bone marrow examination and compared the results to a prior bone marrow examination in the referral center and found an agreement of only 34% for KIT mutation analysis, 68% for cytomorphology, 75% for immunophenotyping and 80% for histopathology [248]. Another retrospective analysis from a German expertise center revealed misdiagnoses in 36% of 140 included patients with advanced systemic mastocytosis [249]. These data are probably representative for the situation in other Western countries and stress the importance of specialized laboratory facilities in order to avoid repeat bone marrow examinations for our patients.

When a person is diagnosed with SM, it is important to correctly counsel them on their prognosis. The risk of anaphylaxis is a major cause of anxiety for patients [52]. This is not surprising since the lifetime prevalence of anaphylaxis among patients with SM is 30-49% [47,48,103]. However, it is difficult to determine who is at risk and who can be reassured. Several clinical characteristics have been demonstrated to be associated with an increased risk for anaphylaxis, such as the absence of skin mastocytosis, male sex, presence of atopy, and an increased total IgE level [77] [50]. Interestingly, there is no linear association between serum tryptase level and the risk on anaphylaxis: the risk is the highest for patients with a moderately increased serum tryptase level [50,158]. Measurement of specific IgE to Hymenoptera venom is also less sensitive in patients with mastocytosis, who often have no detectable or low levels of specific IgE despite experiencing severe anaphylactic reactions [55].

Since anaphylaxis is a potentially life threatening condition, it is unsafe to completely rely on these characteristics when informing an individual patient or determining whom to prescribe adrenalin auto-injectors. Our own efforts to use the basophil activation test (BAT) to assess one's risk on Hymenoptera venom related anaphylaxis proved unsuccessful (Chapter 3.2) [250]. This implies that Hymenoptera venom induced mast cell activation does not happen via IgE in mastocytosis, but for instance through direct binding of venom components to the MRGPRX2 receptor. There have been no publications on the role of MRGPRX2 in Hymenoptera venom-related anaphylaxis at the time of writing. Furthermore, the low sensitivity of the BAT in our study might have been due to technical reasons. Urra et al. recently demonstrated that the identification of basophils by IgE instead of CCR3 (chemokine CC receptor type 3) led to a much higher sensitivity [251]. Another weakness of our study on Hymenoptera venom related anaphylaxis is that component analysis of wasp venom was not performed, although this has become common practice in the years after our BAT study. This was based on a study in Groningen that showed that the sensitivity of sIgE diagnostics among patients with mastocytosis can be enhanced by the measurement of sIgE against components of wasp venom[55]. Moreover, the same study showed that lower cut-off levels should be used for sIgE against wasp venom in patients with mastocytosis specifically [55]. However, it is still unknown whether the presence of sIgE can predict Hymenoptera venom-related anaphylaxis in the future for patients who have not yet experienced anaphylaxis before. This might be a topic that can never be reliably answered, since many factors probably play a role in Hymenoptera venom-related anaphylaxis in mastocytosis, and this can hardly be simulated in an *in vitro* test system.

Organization of care and quality of life

Many aspects of the clinical care for patients with mastocytosis are based on expert opinion. There is a lack of evidence-based guidelines on a national as well as international level. In fact, only one official guideline was published in the U.S.A. in 2018, which mainly focuses on the diag-

nosis of mastocytosis and the treatment of advanced systemic mastocytosis [67]. It is unclear how patients with different subtypes of mastocytosis should be followed up after the diagnosis was made. Too frequent hospital visits will lead to higher medical costs, and might increase the burden of disease on patients. On the contrary, insufficient clinical guidance puts the patient at risk for untreated symptoms and complications. It has been demonstrated in this thesis as well as in other papers that the chance of progression of disease from indolent to advanced systemic mastocytosis is negligible [86,252], although a recent study in the ECNM database revealed that 4.9% of ISM patients showed progression to a more advanced subtype according to WHO criteria [253]. The regular measurement of serum tryptase level is probably sufficient to screen for progression of disease in patients with ISM, since this correlated well to the mast cell load [106]. In line with this, a rise in the serum tryptase level was indicative of an increase in liver and/or spleen size as shown in Chapter 3.3 of this thesis [252]. The D816V allele burden in peripheral blood can also be used for follow-up [44] but is a more expensive assay.

Another important reason for follow-up of patients with ISM is the risk of osteoporosis. In our own cohort, 54% of patients had a decreased bone mineral density, of whom 48% was male (Chapter 2.1) [103]. However, it might not be necessary to perform regular bone mineral density measurements for every patient. For a patient who does not have osteoporosis at diagnosis, the chance of developing osteoporosis in the following years is not increased [254]. Of course, risk factors for osteoporosis such as vitamin D deficiency, menopause and other endocrinological factors should be taken into account when establishing an individual's risk of osteoporosis.

As outlined previously, ISM has an excellent prognosis and does not influence one's life expectancy [39,40,255]. However, the health related quality of life of patients can be significantly influenced. As demonstrated in Chapter 2.2 of this thesis, patients with mastocytosis had a similar perception of their own

health to patients with cancer [229]. The prevalence of psychological symptoms, such as depressive mood and anxiety, was also significantly increased compared with a healthy norm group, but lower than in patients with chronic pain [229]. This supports the hypothesis that the increased prevalence of depression and anxiety in patients with mastocytosis can at least partially be attributed to having a chronic disease with debilitating symptoms, rather than solely the specific effect of mast cell mediators to the brain. As shown in the results of a patient survey by Jennings et al., the unpredictability of symptoms is a major cause of stress for patients [52]. Furthermore, the presence of fatigue can also have a negative influence on one's psychological functioning and overall health-related quality of life. In a small study of 28 patients with SM, a VAS of 53 mm was reported for fatigue compared with 6 mm in healthy controls [63]. However, the range in VAS for fatigue in the mastocytosis group was wide: 15-91 mm[63], illustrating the large variation in symptoms and burden of disease.

Another possible explanation for the increased presence of (subjective) psychological symptoms could be that mast cell mediators such as histamine directly influence brain metabolism. There are two histamine receptors present in the brain: H1R and H3R. Activation of H1R through the binding of histamine helps the body to be active, and leads to increased wakefulness, increased body temperature, cardiac rate, and decreased appetite. Activation of H3R, which is the main present histamine receptor in the brain, has opposite effects and leads to increased sleepiness. Although this has not yet been studied in patients with mastocytosis, high levels of histamine could theoretically influence their mood and quality of sleep through these receptors. Of note, insomnia was one of the domains in the SCL-90 for which our cohort scored significantly higher than healthy controls.

Another relevant neuropeptide is serotonin. Human mast cells produce little to none serotonin but there is evidence that they can express a receptor for serotonin [256]. This neurotransmitter has been linked extensively to psychiatric disorders, mainly depression. Previous research indeed showed an

impaired serotonin metabolism in patients with mastocytosis: two different groups showed that patients with mastocytosis had significantly lower levels of tryptophan and its metabolite serotonin than healthy controls [92,257,258]. Interestingly, there was an association between the level of serotonin and psychiatric symptoms but also fatigue, flushing and diarrhea [257]. The association between low levels of tryptophan and gastrointestinal symptoms was confirmed in another study [259]. We also found a correlation between the SCL-90 score and fatigue and diarrhea (Chapter 2.2), which therefore might be explained by lower levels of serotonin. The decreased levels of serotonin are hypothesized to result from increased conversion of tryptophan into an inactive metabolite kynurenin by the enzyme indoleamine 2,3-dioyxygenase (IDO). The activity of IDO is stimulated by inflammatory cytokines, mainly interferon-ɣ, interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) [259]. These data implicate a possible role for serotonin reuptake inhibitors in the treatment of patients with SM with mood disturbance, but also flushing and gastrointestinal symptoms. Further research is necessary to investigate this hypothesis.

Previous studies showed a much higher rate of psychological problems than our cohort. In a French study, 64% of patients with ISM had symptoms of depression, and 38.6% had some degree of cognitive impairment [65,66]. A similar prevalence of self-reported depression was found in a Dutch and another French cohort [53,91]. We could not confirm the presence of cognitive dysfunction in our own cohort, as results of a Cognitive Failure Questionnaire were comparable to the healthy norm group (data not published). This might be due to the used questionnaire and the study population: the French cohort in which a high rate of cognitive impairment was reported also had high degrees of depression and cognitive dysfunction is a symptom of depression. Furthermore, since mastocytosis has such a heterogeneous clinical picture, different study cohorts can vary significantly. The reproducibility of such studies is further endangered by the often small study cohorts. This stresses the importance of the confirmation of all findings in other cohorts and col-

laboration between expertise centers to form larger and more representative study populations.

Medical procedures and mastocytosis

An important topic for patients with mastocytosis is the risk of iatrogenic anaphylaxis due to medication and invasive procedures. Theoretically, mast cells can be activated by a wide range of triggers including small molecule drugs, physical triggers such as temperature changes, friction and stress. All these factors are present in invasive medical procedures and therefore, it was historically advised to avoid a large list of drugs including most analgesics and radiologic contrast media. These advises are still given to date, leading to unnecessary anxiety in both patients and physicians. Consequently, patients with mastocytosis are sometimes subjected to medieval-style procedures without analgesics because of this fear for anaphylaxis, or on the other hand overtreated with prophylactic drugs to avoid anaphylaxis. They are often advised to avoid NSAIDs, although this is largely based on theoretical grounds. We performed the first prospective study to investigate the risk on NSAID hypersensitivity in patients with mastocytosis (Chapter 4) and found that only 1 out of 50 participants had an objective hypersensitivity reaction to acetylsalicylic acid (ASA), consisting of an urticarial rash that subsided after administration of an oral antihistamine [93] Albeit less reliable, an additional retrospective analysis of the entire cohort of the mastocytosis center was performed, showing a prevalence of 4.1% of self-reported NSAID hypersensitivity reactions. Three out of these 8 patients had anaphylaxis related to an NSAID and needed treatment in an emergency department. Interestingly, 2/8 patients later underwent an NSAID challenge that was negative. It is well know that NSAIDs can aggravate an anaphylactic reaction by acting as a cofactor. There are several possible mechanisms whereby NSAIDs can enhance a reaction caused by mast cell activation. Firstly, they cause increased permeability of gut mucosa, thereby improving allergen availability of mucosal mast cells and antigen presenting cells. Secondly, basophils were proven to be more reactive to allergens in the presence of ASA [260,261]. Similar experiments have not

been published with mast cells yet, probably because it is technically more challenging to obtain live mast cells from a human individual. However, one interesting study using murine mast cells showed a dose-dependent dual effect of ASA on mast cells [262]. At high doses, ASA inhibited all mast cell activity, whereas at an intermediary dose the COX2 expression was increased [262]. Thus patients with mastocytosis who have experienced anaphylaxis, especially (recurrent) iatrogenic anaphylaxis, should still be warned that NSAIDs can worsen mast cell activation. Also, patients with classic risk factors for NSAID hypersensitivity such as asthma or angioedema should avoid COX-1 inhibitors. However, the majority of patients with mastocytosis can safely use NSAIDs.

Next to NSAIDs, invasive medical procedures form another cause of anxiety and uncertainty for patients with mastocytosis. In Chapter 4, a study is presented in which the evidence for iatrogenic anaphylaxis is reviewed [211]. Not surprisingly, there are very few comprehensive studies on this subject. The available evidence largely comes from cohort studies that enlist self-reported reactions, and case reports. From one large retrospective Spanish cohort, the risk on anaphylaxis associated with general anesthesia was 5.4% without premedication and 0.4% with premedication [139]. Although this is higher than in the general population, it should be no reason to avoid anesthesia. The evidence for the type and doses of premedication is also lacking, and recommendations are based on guidelines for radiocontrast allergy. For patients with mastocytosis, the risk on anaphylaxis due to radiocontrast media appeared to be low, and premedication is only advised in patients with previous reactions to radiocontrast media or to other drugs. Again, mechanistic evidence is lacking. One study from the 90's investigated the effects of three kinds of iodine containing contrast media on basophils and mast cells and mainly found that basophils and lung mast cells were activated by the contrast media but skin and gut mast cells were not [263]. To date, other contrast media are used with a lower osmality which should theoretically be less likely to activate basophils or mast cells. However, there are currently no studies published to corroborate this theory. Based on the current knowledge and

experience, radiologic contrast media can safely be administered to patients with mastocytosis without additional measures, except of course those individuals who have a documented radiologic contrast media allergy.

Searching new targets for therapy

Once the diagnosis of mastocytosis is made, the treating physician can encounter the next problem: The lack of effective therapeutic options that have been adequately researched. This is also the case in patients with mastocytosis. Until recently, studies were mainly focused on advanced systemic mastocytosis because of its adverse survival chances. The majority of these studies investigated tyrosine kinase inhibitors (TKI) that target KIT to cause apoptosis of mast cells. For years, the only KIT inhitibitor that was available was imatinib, which is not effective for cells with the common D816V mutation. In 2018, midostaurin was registered, which also inhibits mutated KIT activity [75]. Midostaurin effectively reduces the mast cell load and has greatly enhanced the survival of patients with advanced systemic mastocytosis. Another TKI directed at KIT, avapritinib, showed very promising results in a phase I trials among patients with advanced systemic mastocytosis with 56% reaching complete or partial response [264]. Avapritinib appears to be more specific to D816V mutated KIT which hopefully leads to fewer side effects than midostaurin [265]. These TKI provide no cure for mastocytosis, and when it is interrupted, the mast cells will start proliferating again. Moreover, neoplastic mast cells can become resistant to TKI. Lastly, the TKI mentioned have considerable side effects, mainly intractable nausea, but also myelosuppression and discoloration of hair [75].

For indolent systemic mastocytosis, there are currently no registered drugs available. As stated previously, indolent systemic mastocytosis does not influence one's life expectancy, but its symptoms can cause a significant burden on the quality of life of affected individuals. It would therefore be useful to broaden the array of pharmacological options for patients with benign variants of mastocytosis. Next to well-known mediators such as histamine, eicosanoids and tryptase, mast cells produce many cytokines that are associated

with constitutional symptoms in mastocytosis [7,64,196,197]. It could theoretically be more effective to find one drug that inhibits the release of all these mediators than to combine several drugs that block the actions of these mediators on other cells. One small trial of midostaurin for indolent systemic mastocytosis showed a moderate effect on symptoms, although this was a non-controlled trial, and nausea again was a common side effect [71]. The multi-TKI masitinib was found to diminish mast cell mediator-related symptoms such as fatigue and pruritus in only 8-25% of patients, respectively [70]. A phase II trial with avapritinib for indolent systemic mastocytosis is currently running at the time of writing, and preliminary data show promising effects. Long term results are obviously lacking for all TKI for mastocytosis. Interestingly, the symptoms of systemic mastocytosis greatly overlap with another hematological disease termed Myeloproliferative Neoplasms (MPN). This represents a spectrum of three conditions in which either too many erythrocytes or platelets are being produced, or fibrosis of the bone marrow occurs. In patients with MPN, increased levels of several cytokines such as IL-6 and TNF-α are found to correlate with clinical symptoms [266]. The TKI ruxolitinib, an inhibitor of JAK1 and JAK2, has been proven very effective to reduce the symptom burden of MPN patients in clinical trials [224,225] and is now registered for the treatment of MPN. Interestingly, ruxolitinib also reduced symptoms such as itch and fatigue in two reported patients with systemic mastocytosis [203,204]. For these reasons, ruxolitinib or other JAK-STAT inhibitors might be effective for highly symptomatic patients with systemic mastocytosis, but also in other mast cell related conditions such as mast cell activation syndrome and chronic spontaneous urticaria. We decided to perform an *in vitro* study to investigate the exact effects of ruxolitinib on mast cell activity (chapter 5) [267]. Ruxolitinib appeared to effectively inhibit cytokine production as induced by substance P and the nonspecific pan-activator calcium ionophore (A23187) without causing mast cell apoptosis. However, high concentrations were necessary to achieve statistically significant reduction of degranulation induced by substance P or codeine. It is known that TKI (partly) lose their specificity in high concentrations and it can be questioned whether ruxolitinib still

only inhibits JAK1 and JAK2 activity in such high concentrations [221]. Efforts to prove STAT5 phosphorylation were unsuccessful due to technical difficulties, but incubation of mast cells with the specific STAT5 inhibitor pimozide achieved similar effects on mast cell activation, suggesting a role for STAT5 in cytokine production by mast cells, and to a lesser extent also degranulation.

As with most research, this study generated more questions than answers. Firstly, codeine and substance P were used to induce mast cell degranulation and cytokine production. These were chosen because we were interested in non-IgE mediated mast cell activation, as well as the fact that substance P is an important autologous mediator for pain and itch and plays a role in chronic spontaneous urticaria [268]. Both codeine and substance P are ligands to G-protein coupled receptors (GPCR) such as the MRGPRX2 and neurokinin 1 receptor [13,59,153]. One of the most important intracellular proteins that act downstream of GPCRs is considered to be phosphoinositide 3-kinase (PI3K) and not the JAK-STAT pathway. The fact that ruxolitinib and pimozide did exert an effect on mast cell activation suggests that JAK-STAT must also be involved downstream of GPCR. Indeed, there is some evidence of crosstalk between PI3K and STAT5 in neoplastic mast cells and other myeloid malignancies: activated STAT5 enhances the catalytic domains p85 and p110 of PI3K and enhances the transcription of Akt [218,219]. As expected, when STAT5 metabolism is inhibited, the PI3K/Akt pathway is also less effective [20]. Lastly, it has been shown that the PI3K-Akt cascade is important for IgE mediated mast cell degranulation [269]. Secondly, it must be noted that STAT5 is constitutively active in neoplastic mast cells and that the JAK-STAT metabolism therefore might differ from wild-type mast cells. Theoretically, the JAK activity is decreased by negative feedback loops when STAT5 is continually active. Another possible concern is the fact that in other *in vitro* studies, KIT activation via SCF has shown to inhibit MRGPRX2-induced mast cell degranulation [270]. This implies that interference with proteins that execute KIT's signals intracellularly (i.e. STAT5) could create an opposite effect on mast cell activation on the longer term, if no cytotoxic effect on mast cells is exerted.

Overall, loose bits and pieces of knowledge are present, but data that elucidate the exact roles and interaction of JAK2, STAT5, PI3K and Akt downstream of MRGPRX2 activation are still lacking. Figure 1 depicts a very schematic model of the presumed interaction between the MRGPRX2 receptor and the JAK-STAT pathway as we know now. But before we can confidently expose patients with mastocytosis to JAK-STAT inhibitors, it is important to elucidate the exact functionality and interaction of JAK-STAT and PI3K-Akt, as well as other intracellular routes, downstream of the MRGPRX2 receptor.

Figure 1

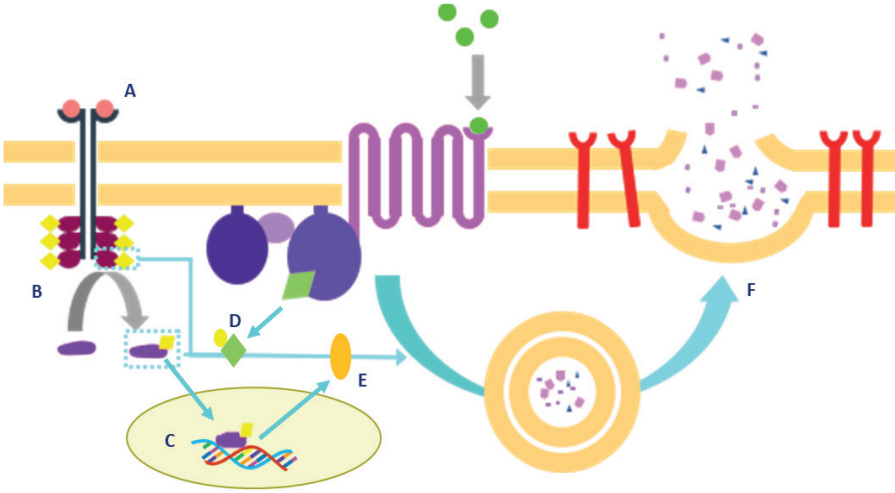


Figure 1. Schematic overview of cooperating receptors. Upon binding of its ligand SCF, the KIT receptor (A) becomes activated and induces phosphorylation of JAK2 (B), which in turns induces phosphorylation of STAT5. Secondly, STAT5 translocates into the cell nucleus to act as a transcription factor (C). STAT5 can probably also enhance the signaling pathway that arises from activation of the MRGPRX2 receptor via stimulating the catalytic domains of PI3K (D) and enhancing transcription of Akt (E) ultimately leading to degranulation and the production of pro-inflammatory mediators (F). Through unknown mechanisms, KIT activation provides negative feedback to MRGPRX2 (G). *Figure adapted from master thesis A.C. van Stigt.*

Conclusion

In this thesis, several myths on mastocytosis were hopefully turned into more factual knowledge.

It can be concluded that the baseline serum tryptase level is not sufficiently sensitive to screen for the presence of systemic mastocytosis, especially in adult patients with mastocytosis in the skin or Hymenoptera venom-related anaphylaxis. However, serum tryptase is a reliable marker for disease progression in patients with proven systemic mastocytosis. Despite its good prognosis, indolent systemic mastocytosis puts a significant burden on the quality of life of patients. The increased prevalence of psychological symp-

toms is probably caused by a combination of the bothersome symptoms and direct effects of mast cell mediators to the brain cells.

Anaphylaxis is an important influence on the quality of life of patients. However, the risk on iatrogenic anaphylaxis is lower than often expected, and NSAIDs and radiologic contrast media can safely be administered to most patients with mastocytosis, hopefully relieving some of the anxiety in many mastocytosis patients. It is still unclear how to predict an individual's risk on anaphylaxis, especially Hymenoptera-induced anaphylaxis.

There are also many questions to be answered about the pathophysiological mastocytosis. Ideally, phenotyping of the neoplastic mast cell clone of an individual would lead to a tailored therapeutic approach. For this purpose, *in vitro* techniques and methods to isolate viable mast cells from the human body should be improved. These translational research methods are probably more representative than *in vitro* experiments with single cell type cultures. Furthermore, little is known about the effects that neoplastic mast cells exert on the rest of the immune system and the local milieu in skin, bone marrow or mucosa. Clarification of those effects could provide better understanding of mastocytosis as a whole and hopefully help to treat patients more accurately.

Research of a rare condition is only possible by cooperating (inter)nationally, since research in small cohorts has important limitations, especially with such a heterogeneous disease. It would also be of great value to share findings beyond the small community of mast cell researchers, since mast cells are most probably important players in other conditions such as fibrosis, auto-immune diseases and cardiovascular disease.

Chapter 7

Summary

7.1 Summary

Due to the heterogeneous nature of mastocytosis, several questions and dilemmas arise in the daily clinical care for patients. A large part of the current practice is based on expert opinion. The aim of this thesis was to elucidate some of these myths and other clinical questions.

In **chapter 1**, an introduction is given on mast cell biology and the definitions, pathophysiology and clinical aspects of mastocytosis.

Chapter 2 starts with a description of the cohort of 136 adult patients with systemic mastocytosis as defined by the WHO. This paper described the variety of clinical symptoms among different subtypes of systemic mastocytosis and provided some interesting findings that led to follow-up research questions. The average delay from the onset of symptoms to diagnosis was 8.1 years. This can be explained by a lack of unawareness for mastocytosis among both physicians and patients. Mastocytosis was recognized earlier in patients with flushing or anaphylaxis than in patients with MPCM or osteoporosis. Furthermore, a difference was detected in clinical phenotype between patients with indolent systemic mastocytosis with or without skin involvement. Those without skin involvement were more often male, and anaphylaxis and osteoporosis were more prevalent in these patients. Of note is that 23.5% of the total cohort had a serum tryptase level <20 μg/L at the time of diagnosis. It can thus be questioned whether this level is the right cut-off as a minor criterium in the WHO criteria. We have elaborated on that question in chapter 3.1 . Furthermore, chapter 2 contains a cross-sectional cohort study in which neuropsychiatric morbidity in mastocytosis was investigated by obtaining a screening questionnaire on psychological symptoms (SCL-90) and the SF-36. Although previous studies had already reported a high prevalence of neuropsychiatric symptoms such as fatigue, depression and cognitive dysfunction in mastocytosis, no-one had yet compared the psychological burden of patients with mastocytosis to patients with other chronic diseases. Our study showed that patients with mastocytosis indeed have more neuropsychiatric

symptoms than healthy controls, but less than patients with chronic pain. Strikingly, patients with mastocytosis had a similarly unfavorable perception of their general health to cancer patients. We concluded that the burden of disease is high in patients with mastocytosis. The increased prevalence of psychological symptoms is probably a consequence of both the impact of physical discomfort as well as the direct effects of mast cell mediators to the brain. It is recommended to evaluate the psychosocial situation of every patient with mastocytosis and to offer adequate counselling if necessary.

In **chapter 3**, several dilemmas in diagnostics are studied. First, we specifically investigated the value of serum tryptase as a screening tool for the presence of systemic mastocytosis. As expected from the previous findings from the cohort study in chapter 2, 28.3% of the 198 patients who were included had a serum tryptase level below the WHO cut-off of 20 μg/L and 10.1% had a serum tryptase level of ≤ 11.4 μg/L which is the international cut-off value for the normal range. Despite the probable biases that apply to this retrospective study, it can be concluded that especially in patients with adult-onset MPCM or Hymenoptera venom-related anaphylaxis, a serum tryptase level ≤ 11.4 is not sufficiently reliable to rule out systemic mastocytosis.
In chapter 3.2, we tried to find a method to identify those patients with mastocytosis who are at risk for Hymenoptera-related anaphylaxis by means of a basophil activation test (BAT). Unfortunately, the BAT was positive in merely one patient of the total cohort of 29, whereas 78% of patients with Hymenoptera-related anaphylaxis had a positive intradermal test and 67% had detectable specific IgE in serum. The BAT therefore was not of additional value to predict Hymenoptera-related anaphylaxis.
A third question that arose from daily practice was whether routinely performed abdominal ultrasonography was useful in the follow-up of patients with indolent systemic mastocytosis. Until 2017, ultrasonography of the abdomen was routinely performed every one to three years to screen for hepatosplenomegaly as a sign of progression of disease. In this study, 88 patients were included and the median follow-up time was 11.2 years. Nine patients with

indolent systemic mastocytosis developed new hepatosplenomegaly, but no-one developed advanced systemic mastocytosis. The increase in liver- and/or spleen size was positively correlated with an increase in serum tryptase level. We thus concluded that routine abdominal radiographic testing is not recommended in patients with indolent systemic mastocytosis and that the absolute risk of progression to advanced subtypes is neglibible. Clinical examination and serum tryptase are sufficient to screen for (the unlikely) progression of indolent to advanced systemic mastocytosis.

Chapter 4 describes two studies on drug-related reactions in mastocytosis. This is a major issue in the daily practice of a mastocytosis clinic and a reason for anxiety in many patients. First, we reviewed the available evidence for iatrogenic anaphylaxis in mastocytosis and found that most recommendations are based on case reports, unsupported hypotheses, or *in vitro* data. The actual risk on iatrogenic anaphylaxis appears to be low in both adults and children with mastocytosis. A moderately increased risk on anaphylaxis of 5.4% was demonstrated for major surgery with general anesthesia. This risk could be decreased to 0.4% by administering adequate pre-medication. Local anesthesia appeared safe. We formulated practical recommendations for perioperative management of both children and adults with mastocytosis, but with the restriction of a lack of reliable clinical evidence.
Chapter 4.2 describes a study in which the prevalence and severity of NSAID hypersensitivity was investigated. Fifty adults with all subtypes of mastocytosis underwent a randomized, placebo-controlled, double-blind provocation test with acetylsalicylic acid. Contrary to popular belief, the rate of hypersensitivity reactions was low. Only one patient experienced an urticarial rash several hours after completion of the provocation test. Three other patients had subjective symptoms, which could theoretically be attributed to mast cell activation but which were very mild. It therefore appeared that NSAIDs can be safely administered to most patients with mastocytosis. In a retrospective study of our entire outpatient cohort with mastocytosis, risk factors for the presence of NSAID hypersensitivity were identified. Extra

caution was advised in patients who have experienced previous hypersensitivity reactions to any kind of drug, and patients with traditional risk factors for NSAID intolerance such as asthma or nasal polyposis.

In **chapter 5**, translational studies are described that are aimed at gaining more insight in the pathophysiological mechanisms of mastocytosis. In chater 5.1, a new possible therapeutic strategy is explored *in vitro*. Ruxolitinib is a JAK1/2 inhibitor that is used in classical myeloproliferative neoplasms. In these patients, it is very effective against itch and constitutional symptoms. Moreover, two case reports of the use of ruxolitinib in systemic mastocytosis confirmed the effects on itch, fatigue and gastrointestinal symptoms. Based on these results, we hypothesized that ruxolitinib would inhibit mast cell degranulation and cytokine production. We studied this *in vitro* using two different mast cell lines which were stimulated with substance P and codeine. Ruxolitinib indeed effectively inhibited mast cell degranulation and cytokine production, although relatively high doses were needed to significantly reduce mast cell degranulation. Inhibition of STAT5 with another compound, pimozide, was also effective to inhibit cytokine production, but only weakly inhibited mast cell degranulation. The JAK-STAT pathway thus appears more important for cytokine production than for degranulation in mast cells.
Chapter 5.2 contains a study in which the percentage of group 2 innate lymphoid cells (ILC2) are investigated in peripheral blood of patients with indolent systemic mastocytosis. This study shows that ILC2s are more abundant in patients with systemic mastocytosis that harbor the D816V KIT mutation. Patients with systemic mastocytosis but without the D816V mutation did not have significantly different ILC2 percentages compared with healthy controls. Given the association between the presence of skin mastocytosis and ILC2 abundance, we hypothesize that the increased numbers of aberrant mast cells in the skin might provide signals that enhance ILC2 migration and proliferation. Of course, more in-depth research is necessary to find a mechanistic explanation for the findings in this study.

Chapter 6 elaborates on the findings described above as well as possible strengths and weaknesses of this thesis. Furthermore, the implications for the daily practice and future research are discussed.

7.2 Samenvatting

De heterogene aard van mastocytose leidt tot diverse vragen en dilemma's omtrent de klinische zorg in de dagelijkse praktijk. Een groot deel van de huidige zorg is gebaseerd op 'expert opinie'. Het doel van dit proefschrift was om enkele van deze mythes te ontrafelen.

Hoofdstuk 1 omvat een introductie over mestcel biologie en de definities, pathofysiologie en klinische aspecten van mastocytose.

Hoofdstuk 2 start met een beschrijving van een cohort van 136 volwassen met systemische mastocytose, zoals gedefinieerd door de WHO. Dit artikel beschrijft de variatie van klinische symptomen tussen de verschillende subtypes van systemische mastocytose en enkele interessante aanvullende bevindingen. Het duurde gemiddeld 8,1 jaar vanaf het begin van symptomen totdat de diagnose systemische mastocytose officieel gesteld werd. Dit is grotendeels te verklaren door de onbekendheid van het ziektebeeld bij zowel artsen als patiënten. Mastocytose werd eerder gediagnosticeerd bij mensen met *flushing* of anafylaxie dan met MPCM of osteoporose. Verder werd er een verschil in klinische fenotype gevonden tussen patiënten met indolente systemische mastocytose met en zonder huid betrokkenheid. Patiënten zonder huid betrokkenheid waren vaker man en anafylaxie en osteoporose kwamen vaker voor in deze groep. Van belang is op te merken dat 23,5% van het totale cohort een serum tryptase waarde <20 µg/L had op het moment van diagnose. Dit is de huidige afkapwaarde als mineur criterium in de WHO criteria, maar gebaseerd op deze bevindingen kan de vraag gesteld worden of dit de juiste afkapwaarde is. In hoofdstuk 3.1 wordt verder ingegaan op deze vraag.
Vervolgens bevat hoofdstuk 2 een studie waarin de neuropsychiatrische morbiditeit van 50 volwassenen met mastocytose werd onderzocht door middel van een screenende vragenlijst over psychologische symptomen (de SCL-90) en de SF-36. Eerder studies toonden reeds een hoge prevalentie aan van neuropsychiatrische symptomen zoals vermoeidheid, depressie en cognitieve dysfunctie. Echter werd in geen enkele eerdere studie de groep met mastocy-

tose vergeleken met patiënten met andere chronische aandoeningen. Onze studie toonde aan dat patiënten inderdaad meer neuropsychiatrische symptomen hebben dan gezonde controles, maar minder dan patiënten met chronische pijn. Opvallend genoeg was de perceptie van de algemene gezondheid onder de patiënten met mastocytose gelijk aan die van de patiënten met kanker. Alhoewel onze studie geen pathofysiologische mechanismen heeft onderzocht, lijkt de toename van psychologische symptomen waarschijnlijk het gevolg van zowel de impact van fysieke klachten als de directe effecten van mestcel mediatoren op het brein. Het wordt aanbevolen om de psychosociale situatie van elke patiënt met mastocytose te evalueren en zo nodig begeleiding te bieden.

In **hoofdstuk 3** worden diverse dilemma's omtrent diagnostiek bestudeerd. Hoofdstuk 3.1 beschrijft een retrospectief onderzoek naar de waarde van het serum tryptase als screenend instrument voor aanwezigheid van systemische mastocytose. Zoals verwacht naar aanleiding van de bevindingen uit de cohort studie in hoofdstuk 2.1, had 28,3% van de 198 patiënten in deze vervolg studie een serum tryptase onder de WHO afkapwaarde van 20 µg/L en 10,1% had een serum tryptase gehalte ≤ 11.4 µg/L welke de internationale normaalwaarde is. Uiteraard kent een retrospectieve studie een groot risico op inclusie bias en is dit niet de beste methode voor het onderzoeken van een screenend instrument. Echter kan wel geconcludeerd worden dat het serum tryptase onvoldoende betrouwbaar is voor het screenen op systemische mastocytose bij patiënten met MPCM die na de puberteit zijn ontstaan of Hymenoptera gerelateerde anafylaxie.
In hoofdstuk 3.2 werd onderzocht of de basofiel activatie test (BAT) een goede screenende assay is voor het voorspellen van Hymenoptera gerelateerde anafylaxie bij mensen met mastocytose. De BAT was echter positief in slechts één patiënt van de totale groep van 29, terwijl zeven van de negen patiënten met Hymenoptera gerelateerde anafylaxie wel een positieve huidtest hadden en zes van de negen hadden detecteerbaar specifiek IgE tegen wespengif. De BAT bleek dus een onbetrouwbare test voor het identificeren van diegenen

die risico lopen op Hymenoptera gerelateerde anafylaxie.
In hoofdstuk 3.3 wordt de waarde van routinematig verrichte echografie van de buik voor het detecteren van progressie van indolente naar agressievere vormen van systemische mastocytose onderzocht. Tot 2017 werd in het Erasmus MC bij deze patiënten standaard elke één tot drie jaar een echo abdomen verricht, maar de vraag rees of dit zinvol was. Er werden 88 patiënten met systemische mastocytose geïncludeerd en de mediane follow-up duur was 11,2 jaar. Negen van de patiënten ontwikkelde nieuwe hepato- en/of splenomegalie, maar niemand ontwikkelde agressieve mastocytose of een bijkomende hematologische neoplasie. Het serum tryptase was gecorreleerd met een toename van lever- en/of milt grootte. Het risico op progressie van indolente systemische mastocytose op zichzelf bleek verwaarloosbaar. Derhalve concludeerden wij dat jaarlijks lichamelijk onderzoek en serum tryptase gehalte voldoende zijn voor het screenen naar progressie van ziekte bij mensen met indolente systemische mastocytose.

Hoofdstuk 4 omvat twee studies naar medicijn gerelateerde overgevoeligheidsreacties bij mastocytose. Dit is een belangrijke kwestie in de dagelijkse praktijk van een mastocytose kliniek en een reden voor onzekerheid bij patiënten. Hoofdstuk 4.1 is een review naar het bewijs voor iatrogene anafylaxie bij mastocytose. Hieruit blijkt dat de meeste aanbevelingen voor gebruik van medicatie, met name pijnstillers en anesthetica, voortkomen uit casus beschrijvingen, ongefundeerde theoretische hypotheses of *in vitro* data. Het feitelijke risico op iatrogene anafylaxie blijkt zowel bij kinderen als volwassenen met mastocytose laag te zijn. Voor algehele narcose is het risico wel bewezen hoger dan in de algemene bevolking, namelijk 5,4% in één grote studie. Dit risico kon verlaagd worden naar 0,4% door toedienen van premedicatie. In ons review formuleerden wij aanbevelingen voor perioperatief management van mensen met mastocytose, alhoewel opnieuw gebaseerd op beperkt wetenschappelijk bewijs.
In hoofdstuk 4.2 wordt het risico op NSAID gerelateerde hypersensitiviteit onderzocht. Vijftig volwassenen met mastocytose ondergingen een dubbel-

blinde, placebo-gecontroleerde provocatie met acetylsalicylzuur. In tegenstelling tot wat meestal gedacht wordt, was het aantal patiënten met een positieve provocatie laag: Slecht één patiënt ontwikkelde een urticariële uitslag enkele uren na afronding van de provocatie en drie anderen hadden subjectieve symptomen die mogelijk toe te schrijven zijn aan mestcel activatie. Alle reacties waren mild van aard. Hieruit kan geconcludeerd worden dat NSAID's veilig kunnen worden voorgeschreven aan de meeste mensen met mastocytose. In een retrospectieve analyse van het gehele Erasmus MC cohort van volwassenen met mastocytose werden vervolgens een aantal risicofactoren voor NSAID hypersensitiviteit geïdentificeerd. Extra voorzichtigheid wordt aanbevolen bij mensen die eerder overgevoeligheidsreacties hebben gehad op andere medicamenten en patiënten met de traditionele risicofactoren voor NSAID hypersensitiviteit zoals astma en neuspoliepen.

In **hoofdstuk 5** worden twee translationele studies beschreven. In hoofdstuk 5.1 werd een mogelijke nieuwe medicamenteuze behandeling voor mastocytose onderzocht *in vitro*. Ruxolitinib is een JAK1/2 remmer die is geregistreerd voor behandeling van myeloproliferatieve neoplasmata. In deze patiënten werkt ruxolitinib erg goed tegen o.a. jeuk en constitutionele symptomen. Bovendien zijn er twee casus beschrijvingen gepubliceerd waarin ruxolitinib werd gebruikt voor de behandeling van systemische mastocytose en waarin het gunstige effect op jeuk, vermoeidheid en gastro-intestinale symptomen werd bevestigd. Gebaseerd op deze gegevens vermoedden wij dat remming van de JAK-STAT route effectief kon zijn voor het remmen van mestcel degranulatie en cytokine productie. Dit werd bestudeerd in twee verschillende mestcel lijnen die werden gestimuleerd met substance P en codeïne. Incubatie van de mestcellen met ruxolitinib remde inderdaad de mestcel degranulatie en cytokine productie, alhoewel relatief hoge doses nodig waren voor het bereiken van een statistisch significant effect. Remming van STAT5 met pimozide leidde ook tot afname van cytokine productie maar had geen significant effect op de mestcel degranulatie. De JAK-STAT route is derhalve mogelijk belangrijker voor de cytokine productie van voor mestcel degranulatie, al-

hoewel vervolg studies nodig zijn voor het beter interpreteren van deze data. In hoofdstuk 5.2 werd het gehalte “group 2 innate lymphoid cells” (afgekort ILC2s) in perifeer bloed van patiënten met indolente systemische mastocytose onderzocht. Dit onderzoek toonde aan dat ILC2s verhoogd aanwezig zijn in bloed van patiënten met mastocytose die de D816V KIT mutatie hebben. Het ILC2 percentage van patiënten met mastocytose maar zonder D816V mutatie was niet significant verschillend van gezonde controle personen. Aangezien er ook een associatie gevonden werd tussen het ILC2 percentage en aanwezigheid van huid mastocytose, veronderstellen wij dat het toegenomen aantal afwijkende mestcellen in de huid van deze patiënten signalen verspreiden die leiden tot toegenomen migratie en proliferatie van ILC2s. Vervolg onderzoek is noodzakelijk om de precieze interactie tussen (afwijkende) mestcellen en ILC2s verder te onderzoeken.

In **hoofdstuk 6** wordt verder ingegaan op de betekenis en beperkingen van bovenstaande onderzoeken en de mogelijke implicaties voor zowel de klinische praktijk als toekomstig onderzoek.

Chapter 8

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8.2 Co-authors and affiliations

Author	Affiliation
Benjamin J. Schrijver BSc	Laboratory of Medical Immunology, Department of Immunology, Erasmus University Medical Center
C. Louk de Mol BSc	Medical student, faculty of medicine, Erasmus University
Conny C.P.A. van Holten-Neelen BSc	Laboratory of Medical Immunology, Department of Immunology, Erasmus University Medical Center
Esmee K. van der Ploeg Msc	Department of Pulmonary Medicine, Erasmus MC, Rotterdam, The Netherlands Department of Cell Biology, Erasmus MC, Rotterdam, The Netherlands
Jan A.M. van Laar MD PhD UHD	Department of Internal Medicine, Section of Allergy & Clinical Immunology, University Medical Center Laboratory of Medical Immunology, Department of Immunology, Erasmus University Medical Center
Marco W.J. Schreurs PhD	Laboratory of Medical Immunology, Department of Immunology, Erasmus University Medical Center
Marie-Roos Vermeiren BSc	Medical student, faculty of medicine, Erasmus University
Mark J.A. Rietveld BSc	Medical student, faculty of medicine, Erasmus University
Nicolette J.T. Arends MD PhD	Department of Pediatrics, Section of Allergy, Sophia Children's Hospital
Paul L.A. van Daele MD PhD UHD	Department of Internal Medicine, Section of Allergy & Clinical Immunology, University Medical Center Laboratory of Medical Immunology, Department of Immunology, Erasmus University Medical Center
Prof. Hanneke C. Kluin-Nelemans MD PhD	Department of Hematology, University Medical Center Groningen
Prof. P. Martin van Hagen MD PhD	Department of Internal Medicine, Section of Allergy & Clinical Immunology, University Medical Center Laboratory of Medical Immunology, Department of

	Immunology, Erasmus University Medical Center
Ralph Stadhouders PhD	Department of Pulmonary Medicine, Erasmus MC, Rotterdam, The Netherlands Department of Cell Biology, Erasmus MC, Rotterdam, The Netherlands
Prof. Roy Gerth van Wijk MD PhD	Department of Internal Medicine, Section of Allergy, Erasmus MC
Prof. Suzanne G.M.A. Pasmans MD PhD	Department of Dermatology, Sophia Children's Hospital and University Medical Center
Sophie Q.A. van der Vet BSc	Medical student, faculty of medicine, Erasmus University
Vincent H.J. van der Velden PhD	Laboratory of Medical Immunology, Department of Immunology, Erasmus University Medical Center
Virgil A.S.H. Dalm MD PhD	Department of Internal Medicine, Section of Allergy & Clinical Immunology, University Medical Center Laboratory of Medical Immunology, Department of Immunology, Erasmus University Medical Center
Willem A. Dik PhD	Laboratory of Medical Immunology, Department of Immunology, Erasmus University Medical Center

8.3 Abbreviations

A23187	Calcium ionophore
AdvSM	Advanced systemic mastocytosis: common denominator for all aggressive subtypes
ALAT	Alanine aminotransferase
ASA	Acetylsalicylic acid
ASAT	Aspartate aminotransferase
ASM	Agressive systemic mastocytosis
BAT	Basophil activation test
BM	Bone marrow
CCR3	Chemokine CC receptor type 3
CM	Cutaneous mastocytosis
ECNM	European Competence Network for Mastocytosis
ESR	Erythrocyte sedimentation rate
FcεR1	IgE receptor type 1
GPCR	G-protein coupled receptor
H1R	Histamine 1 receptor
H3R	Histamine 3 receptor
HMC1	Human mast cell 1 (cell line)
HVA	Hymenoptera venom-related anaphylaxis
IDT	Intradermal test
IgE	Immunoglobulin E
IL-6	Interleukin 6
ILC2	Group 2 innate lymphoid cells
ILCP	Innate lymphoid precursor
IQR	Interquartile range
ISM	Indolent systemic mastocytosis
ISM s-	ISM without skin involvement
ISM s+	ISM with skin involvement
JAK1	Janus kinase 1
JAK2	Janus kinase 2
LAD2	Laboratory of allergic diseases type 2 (cell line)

MC	Mast cell
MCL	Mast cell leukemia
MCP-1	Monocyte chemoattractant protein 1
MIS	Mastocytosis in the skin
MPCM	Maculopapular cutaneous mastocytosis (formerly known as urticaria pigmentosa)
MRGPRX2	Mas-related G-protein coupled receptor 2
NSAID	Non-steroidal anti-inflammatory drug
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PI3K	Phosphoinositide 3-kinase
RQ-PCR	Real time quantitative polymerase chain reaction
SCF	Stem cell factor
SCL-90	Symptom checklist 90
SD	Standard deviation
SF-36	36-item short form survey
sIgE	Specific IgE to one (component of an) allergen
SM	Systemic mastocytosis
SM-AHN	Systemic mastocytosis with associated hematological neoplasm
SSM	Smouldering systemic mastocytosis
STAT5	Signal transducer and activator of transcription 5
Th2	Type 2 T helper cell
TKI	Tyrosine kinase inhibitor
TNFα	Tumor necrosis factor alpha
VAS	Visual analogue scale
WHO	World health organization

Chapter 9

PhD portfolio and Curriculum Vitae

9.1 PhD portfolio

Name: Maud Hermans

Erasmus MC Department: Internal medicine,

Section Allergy & Clinical Immunology

Research School: Molecular Medicine

Promotors: Prof. Dr. R. Gerth van Wijk & Prof. Dr. P.M. van Hagen

Supervisors: Dr. P.L.A. van Daele & Dr. W.A. Dik

	Year	ECTS
Courses		
Evidence Based Medicine	2015	0,5
Medical Ethics	2015	0,3
Basic Immunology Course Groningen	2016	0,6
BROK	2016	0,8
FOCIS European Advanced Immunology Course	2016	0,9
Workshop coaching of medical students (x2)	2016, 2017	0,5
Immunology course for residents, Erasmus MC	2017	1,0
Excel course	2017	0,3
Introduction to Data Analysis	2017	1
Open Clinica database management	2019	0,3
Seminars and workshops		
EAACI exam meetings 4-6x per year	2016 - 2018	1,5
Two workshops on coaching of medical students	2016, 2017	0,2
Workshop 'Teaching of groups'	2018	0,2
Workshop 'Interactive teaching methods'	2019	0,1
Workshop 'Docent professionalisering'	2020	0,4
Presentations		
Patient society for mastocytosis NL	2017	0,5
EAACI, Helsinki (2 posters, 1 presentation)	2017	1
Research presentation MPN working group	2017	0,5
EAACI, Munich (1 presentation)	2018	0,5
Patient educational meetings for mastocytosis, Erasmus MC (3x)	2016 - 2020	2
National symposium Myeloproliferative Neoplasms	2018	0,5
NVVA conference	2019	0,5
Science day Erasmus Internal Medicine (poster)	2019	0,5
Regionale refereeravond Allergologie & Immunologie, Rotterdam (2x)	2019	1
EMBRN, Uppsala (poster presentation)	2019	0,5
European Hematology Association, Amsterdam	2019	1
Symposium patient society for MPN NL	2019	0,5
Research meeting Laboratory of Medical Immunology Erasmus MC	2020	0,5

(Inter)national conferences attended		
NIV conference Maastricht	2015, 2016	2
Component Resolved Diagnostics in Allergology	2015	0,3
NVVA conferences (x5)	2016 - 2019	2
Rotterdam Internal Medicine day symposium (x2)	2016, 2017	2
National symposium Allergy & Clinical Immunology, Rotterdam (x4)	2015 - 2019	1,2
Symposium Mast cells And Urticaria, Paris	2015	1,0
Opening symposium Mastocytosis Centre, Groningen	2016	0,3
Science days Erasmus Internal Medicine (x2)	2017, 2019	2
EMBRN, Prague	2017	1
EAACI, Helsinki	2017	1
EAACI, München	2018	1
European Competence Network Mastocytosis conference, Paris	2017	1
European Competence Network Mastocytosis conference, Salerno	2018	1
National Conference on Mastocytosis, Groningen	2019	0,3
EMBRN, Uppsala	2019	1
EAACI, London *digital version*	2020	0,3
Other		
Peer-reviewing for journals		
Internal Medicine Journal	2016	0,3
Clinical & Experimental Allergy	2016	0,3
Calcified Tissue International	2016	0,3
Journal of Allergy & Clinical Immunology (2 manuscripts)	2017	0,6
Clinical Pharmacology: Advances and Applications	2019	0,3
Acta Haematologica	2020	0,3
Respiratory Research	2020	0,3
Teaching		
Clinical Reasoning classes for Bachelor students	2015 - 2017	2
Supervision of clinical interns in internal medicine	2015 - 2018	2
Coaching of medical bachelor students	2016 - 2019	1
Supervision of yearly systematic review project for Bachelor students	2018 - 2020	1
Supervision of students in scientific projects		
Mark Rietveld	2015	1
Louk de Mol	2016	1
Sophie van der Vet	2017	1
Marie Roos Vermeiren	2018 - 2019	1
Jan Sakoltchik	2018 - …	1
Astrid van Stigt	2018 - 2019	1
Liselotte Jeletich & Bas Munting	2019 - …	1
Lecturing		

Dermatology residents	2017	0,3
Lecturing of medical Bachelor and Master students on various occasions and subjects	2016 - …	2
Immunology lecturing for residents on various occasions	2018 - …	0,5
Lecture on mastocytosis for laboratory staff	2018	0,3
Allergology lecturing Master students 5x per year	2018 - …	2
Acute Medicine residents	2020	0,3
Total ECTS		**67,3**

9.2 List of publications

Accepted Increased group 2 innate lymphoid cells in peripheral blood of adults with mastocytosis.
E.K. van der Ploeg, M.A.W. Hermans, V.H.J. van der Velden, W.A. Dik, P.L.A. van Daele, R. Stadhouders
Journal of Allergy and Clinical Immunology

Accepted HLH caused by a HSV-2 infection, a case report and review of the literature
E.M. Jongbloed, M.A.W. Hermans, M. Wabbijn, J.J.A. van Kampen, J.A.M. van Laar
Netherlands Journal of Medicine

July 2020 Are Patients at Risk for Recurrent Disease Activity After Switching From Remicade® to Remsima®? An Observational Study.
L. Xue, K. van Bilsen, M.W.J. Schreurs, M.E.J. van Velthoven, T.O. Missotten, A.A.H.J. Thiadens, R.W.A.M. Kuijpers, P. van Biezen, V.A.S.H. Dalm, J.A.M. van Laar, M.A.W. Hermans, W.A. Dik, P.L.A. van Daele, P.M. van Hagen
Frontiers in Medicine.

Apr 2020 Psychological functioning and quality of life in patients with mastocytosis: a cross-sectional study.
M.R. Vermeiren, L. W. Kranenburg, P.L.A. van Daele, R. Gerth van Wijk, M.A.W. Hermans
Annals of Allergy Asthma and Immunology

Nov 2019 Indolente systemische mastocytose, zich presenterend als een anafylactische reactie op meerdere klassen antibiotica.
G.H.J. Rösken, M.S. van Maaren, M.A.W. Hermans
Nederlands Tijdschrift voor Astma, Allergie en Immunologie

Oct 2019 Listeria infection on patients using anti-TNF-a treatment: Should

there be preventive strategies?
B.P. Krijthe, M.A.W. Hermans, C.A.M. Schurink, P.L.A. van Daele
European Journal of Internal Medicine

July 2019 Systemic mastocytosis with normal serum tryptase: Rule or exception?
M.A.W. Hermans, M.W.J. Schreurs, P.L.A. van Daele
Journal of the European Academy of Dermatology and Venereology

June 2019 Mast cells in cardiovascular disease: From bench to bedside.
M.A.W. Hermans, J. Roeters van Lennep, P.L.A. van Daele P.L.A., I. Bot
International Journal of Molecular Sciences

Apr 2019 A puzzling haptoglobin level in a patient who is treated with tocilizumab.
M.A.W. Hermans, P.L.A. van Daele
Netherlands Journal of Medicine

June 2018 The JAK1/JAK2- inhibitor ruxolitinib inhibits mast cell degranulation and cytokine release.
M.A.W. Hermans, B. Schrijver, J.C.P.A. van Holten-Neelen, R. Gerth van Wijk, P.M. van Hagen, P.L.A. van Daele, W.A. Dik
Clinical and Experimental Allergy

Mar 2018 Nonsteroidal anti-inflammatory drug hypersensitivity: Not always an allergy!
M.A.W. Hermans, R. Otten, A.F. Karim, M.S. van Maaren
Netherlands Journal of Medicine

Mar 2018 Low frequency of acetyl salicylic acid hypersensitivity in mastocytosis: the results of a double-blind, placebo-controlled chal-

lenge study.
M.A.W. Hermans, S.Q.A. de Vet, P.M. van Hagen, R. Gerth van Wijk, P.L.A. van Daele
Allergy

Jan 2018 Scleroderma-like renal crisis in a patient with anti-threonyl-tRNA synthetase-associated antisynthetase syndrome.
M.A.W. Hermans, J.R. Miedema, R.M. Verdijk, P.L.A. van Daele
Rheumatology

Jan 2018 IgG4-gerelateerde ziekte: huidige stand van zaken.
A.F. Karim, M.A.W. Hermans, R.M. Verdijk, P.M. van Hagen, J.A.M. van Laar
Nederlands Tijdschrift voor Astma en Allergie

Aug 2017 Fotoquiz: Een zeldzame oorzaak van insufficiëntie fracturen.
M.A.W. Hermans, P.L.A. van Daele
Nederlands Tijdschrift voor Geneeskunde

Aug 2017 Management around invasive procedures in mastocytosis.
M.A.W. Hermans, N.J.T. Arends, R. Gerth van Wijk, P.M. van Hagen, H.C. Kluin-Nelemans, J.N. Oude Elberink, S.G.M.A. Pasmans, P.L.A. van Daele
Annals of Allergy Asthma and Immunology

May 2017 Abdominal ultrasonography has limited value in the care for patients with indolent systemic mastocytosis.
C.L. de Mol, M.A.W. Hermans, R. Gerth van Wijk, P.M. van Hagen, P.L.A. van Daele
Hematology

Oct 2016 A unique presentation of pulmonary disease in advanced sys-

temic mastocytosis,proven by the presence of mast cells in broncheoalveolar lavage.
M.A.W. Hermans, A. Broijl, P.L.A. van Daele
Journal of Medical Case Reports

Apr 2016 Klinische Les: Systemische Mastocytose.
M.A.W. Hermans, M. Verburg, J.A.M van Laar, P.M. van Hagen, S.G.M.A. Pasmans, P.L.A. van Daele
Nederlands Tijdschrift voor Geneeskunde

Apr 2016 The Basophil Activation Test Is Not a Useful Screening Tool for Hymenoptera Venom-Related Anaphylaxis in Patients with Systemic Mastocytosis.
M.J.A. Rietveld, M.W.J. Schreurs, R. Gerth van Wijk, P.L.A. van Daele, M.A.W. Hermans
International Archives of Allergy and Immunology

Feb 2016 Systemic mastocytosis: A cohort study on clinical characteristics of 136 patients in a large tertiary centre.
M.A.W. Hermans, M.J.A. Rietveld, J.A.M. van Laar, V.A.S.H. Dalm, M. Verburg, S.G.M.A. Pasmans, R. Gerth van Wijk, P.M. van Hagen, P.L.A. van Daele.
European Journal of Internal Medicine

Oct 2015 Predictive Accuracy and Feasibility of Risk Stratification Scores for 28-day Mortality of
Patients with Sepsis in an Emergency Department.
M.J.M. Hilderink, A.A. Roest, M.A.W. Hermans, Y.C. Keulemans, C.D.A. Stehouwer, P.M. Stassen
European Journal of Emergency Medicine

May 2014 Two patients with a neuroendocrine tumor of the small intestine and paraneoplastic myasthenia gravis.
M.A.W. Hermans, B.M.L. Stelten, H.R. Haak, W.W. de Herder, M.W. Dercksen
Case Reports in Endocrinology, Diabetes and Metabolism

Sept 2011 Identification of patients with upper gastrointestinal bleeding who do not need immediate treatment.
L.M. Jansen, P. Leffers, M.A.W. Hermans, P.M. Stassen, A. Masclee, Y.C. Keulemans
Netherlands Journal of Medicine

Apr 2011 The Value of the Mortality in Emergency Department Sepsis (MEDS) score, C reactive protein and lactate in predicting 28-day mortality of sepsis in a Dutch emergency department.
M.A.W. Hermans, P. Leffers, L.M. Jansen, Y.C. Keulemans, P.M. Stassen
Emergency Medical Journal

9.3 Curriculum vitae

Maud Hermans was born on December 28th 1986 in Weert. After finishing VWO at the Philips van Horne Gemeenschap in Weert, she started medical school at Maastricht University in 2004. She became interested in clinical immunology during the internal medicine internship in Maastricht University Medical Center. She graduated in 2010 and after four months of traveling, she started working as a senior house officer on the internal medicine department in Maxima Medisch Centrum, Veldhoven. In august 2010, she started with the specialization of internal medicine under supervision of Prof. dr. Harm Haak, and worked in the MMC for another three years. In 2014, she moved to Rotterdam for the second half of the specialization and especially the subspecialty of Allergy & Clinical Immunology in the Erasmus Medical Center under supervision of dr. Paul van Daele. The specialization was finished in October 2018. Meanwhile, she started a PhD project on mastocytosis in 2015. The results of this research are presented in this thesis. Together with other colleagues in the Netherlands and the patient society, she has formed a national network for mastocytosis which aims at improving the care for patients with mastocytosis.