

MR. THOMAS KRISTENSEN (Orcid ID : 0000-0002-9834-8771)
DR. CHARLOTTE G. MORTZ (Orcid ID : 0000-0001-8710-0829)

Article type : Original Article: Biotechnology and in vitro Diagnostics

Title Page

Title

Prospective evaluation of the diagnostic value of sensitive *KIT* D816V mutation analysis of blood in adults with suspected systemic mastocytosis

Short title

Identification of SM by sensitive *KIT* D816V analysis of PB

Authors

Thomas Kristensen, PhD¹

Hanne Vestergaard, MD, PhD²

Carsten Bindslev-Jensen, MD, PhD, DMSci^{3,4}

Charlotte Gotthard Mortz^{3,4}

Henrik Fomsgaard Kjaer, MD, PhD^{3,4}

Markus Ollert, MD, DMSci^{4,5}

Michael Boe Møller, MD, DMSc¹

Sigurd Broesby-Olsen, MD^{3,4}

on behalf of the Mastocytosis Centre Odense University Hospital (MastOUH)

¹Department of Pathology, Odense University Hospital, Odense, Denmark

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13187

This article is protected by copyright. All rights reserved.

²Department of Hematology, Odense University Hospital, Odense, Denmark

³Department of Dermatology and Allergy Centre, Odense University Hospital, Odense, Denmark

⁴Odense Research Center for Anaphylaxis (ORCA), Odense University Hospital, Odense, Denmark

⁵Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg

Correspondence

Thomas Kristensen, Department of Pathology, Odense University Hospital, Winsloewparken 15, DK-5000 Odense C, Denmark. Phone (+45)6541 1660. E-mail: thomas.kielsgaard.kristensen@rsyd.dk

Funding:

None

Key Words

Anaphylaxis, insect venom allergy, *KIT* D816V mutation, systemic mastocytosis, urticaria pigmentosa

Abstract

Background: Sensitive *KIT* D816V mutation analysis of blood has been proposed to guide bone marrow (BM) investigation in suspected systemic mastocytosis (SM). The aim of this prospective study was for the first time to compare the D816V-status of the “screening blood sample” used to guide BM biopsy in suspected SM to the outcome of the subsequent BM investigation.

Methods: 58 adult patients with suspected SM were included. The outcome of sensitive *KIT* D816V-analysis of blood was compared to the result of the BM investigation.

Results: Screening blood samples from 44 of 58 patients tested D816V-positive. In 43 of these, SM was subsequently diagnosed in the BM investigation. One patient with a D816V-positive screening sample was diagnosed with monoclonal MC activation syndrome.

Screening blood samples from 14 patients tested D816V-negative. SM was subsequently diagnosed in five of these, whereas nine patients did not fulfil any diagnostic SM criteria (excluding tryptase-criterion). Of the 48 SM patients, 90% tested D816V-positive. Thirteen SM patients presented with hymenoptera venom-induced anaphylaxis, no skin lesions and baseline serum-tryptase ≤ 20 ng/mL. Of these, 92% tested D816V-positive in the screening blood sample.

Conclusion: This prospective study demonstrates that a D816V-positive result in a screening blood sample identifies SM among patients with hymenoptera venom-induced anaphylaxis in whom the diagnosis would most probably have been missed, with potential severe implications. The observed false negative screening results also underline that BM investigation is mandatory in all adult patients with clear signs of, or highly suspected SM, regardless of the *KIT* mutation status.

Introduction

Mastocytosis is a heterogeneous group of diseases characterized by growth and accumulation of neoplastic mast cells (MCs) (1-3). Systemic mastocytosis (SM) often represents a diagnostic challenge, both in the clinic where symptoms may be variable and non-specific and in the laboratory where pathoanatomical changes may be subtle (4). A significant fraction

of SM patients lack the characteristic dense MC infiltrates in the bone marrow (BM) (5). In these patients, the BM infiltration is very discrete and the SM diagnosis therefore solely based on WHO minor diagnostic criteria (referred to as “occult mastocytosis”) (6). In the clinic, it may be difficult to identify the patients at the highest risk of having SM, particularly in the absence of characteristic mastocytosis skin lesions (7). An invasive BM investigation may therefore only be performed in patients with specific symptoms and a strong clinical suspicion of SM. Altogether, this may lead to a significant fraction of patients with SM not being correctly diagnosed and treated which may have severe implications. SM patients have an increased risk of severe and unpredictable anaphylaxis (7-9), and SM patients undergoing venom immunotherapy should be treated life-long, since fatal reactions after discontinuation have been reported (10). SM is furthermore associated with increased risk of comorbidities, such as osteoporosis, and establishing a correct diagnosis of SM is therefore important for early detection and prophylaxis (9, 11).

The currently most widely used laboratory test in suspected SM is baseline serum total tryptase (hereafter referred to as “tryptase”). The tryptase level is often elevated in patients with SM (12). However, it is recognized that tryptase levels may also be elevated in differential diagnostic conditions (12). Further, a significant number of SM patients have tryptase levels ≤ 20 ng/mL (5, 13, 14), or even within the normal range (< 11.4 ng/mL) (15), thus limiting both the sensitivity and the specificity of tryptase measurements as a diagnostic test in suspected SM.

The vast majority of adults with mastocytosis carry the activating somatic *KIT* D816V mutation (16-18). Recent technical advances now allow detection of the mutation in blood in most patients, including indolent SM (ISM) (14, 19). Sensitive *KIT* D816V mutation analysis of blood has therefore been proposed as a tool to guide BM investigation in suspected SM (20), and has been included in recent consensus diagnostic algorithms (21). We recently

validated this approach in patients with anaphylaxis and identified patients with SM on the basis of a D816V-positive blood sample (22). We therefore now systematically perform *KIT* D816V mutation analysis of blood as a diagnostic test in patients with suspected SM at the Mastocytosis Centre Odense University Hospital (MastOUH). The test is performed in all patients with mastocytosis skin lesions, hymenoptera venom anaphylaxis (irrespective of tryptase level), unexplained anaphylaxis and/or diffuse, fluctuating symptoms compatible with MC activation. Patients testing D816V-positive are routinely offered a full BM investigation. A full BM investigation may also be performed to confirm, or rule out, a suspicion of SM in patients testing D816V-negative in blood, in the presence of other markers of SM, such as mastocytosis skin lesions, and/or other typical clinical symptoms indicative of SM.

Here, we present the first prospective study in which the result of sensitive *KIT* D816V mutation analysis of the “screening blood sample” used to guide BM biopsy in suspected SM is compared to the final outcome of the subsequent BM investigation. The study demonstrates that sensitive mutation analysis of blood allows identification of a significant number of SM patients with no skin lesions and normal tryptase levels, in particular among patients with hymenoptera venom-induced anaphylaxis.

Methods

Patients and study design

A total of 58 patients with suspected SM were included in the study. Included patients fulfilled the following criteria; i) a BM investigation was performed at MastOUH between April 9th 2014 and December 15th 2016 because of clinical suspicion of SM, ii) no previous BM biopsy was performed, and iii) a screening blood sample was analyzed for the *KIT* D816V mutation as part of the routine diagnostic work-up of suspected SM prior to BM

examination. All patients fulfilling criteria i) and ii) also fulfilled criteria iii) and the included patients thus represent an entire hospital based case-series of suspected SM. The main presenting symptoms of the 58 patients were the following; mastocytosis skin lesions (in some patients also anaphylaxis and/or elevated tryptase level) (N=26), hymenoptera venom anaphylaxis (in some patients also elevated tryptase level) (N=18), anaphylaxis with unknown elicitor or elicitor other than hymenoptera venom (in some patients also elevated tryptase level) (N=9), elevated tryptase level (>20 ng/mL) (N=3), chronic urticaria (N=1), gastrointestinal symptoms and flushing (N=1). See **Supplementary Table S1** for main characteristics of each patient.

None of the patients have been included in any previous MastOUH-study. All patients were >18 years of age. The study was performed in accordance with the National Committee on Health Research Ethics and the Helsinki Declaration.

KIT D816V mutation analysis

KIT D816V mutation analysis of gDNA from blood or BM total leukocytes was performed using a quantitative mutation-specific qPCR assay with a high analytical sensitivity (hence the term “sensitive *KIT* D816V mutation analysis”) previously described in detail (23). The qPCR data analysis was performed using recently published refined criteria for positivity (14). These previous studies from our center describe the validation of the qPCR method, including analysis of multiple *KIT* D816V mutation-negative control samples. We therefore consider the method to be validated from a technical perspective, and apart from the negative and positive control samples included in every qPCR run for quality control in the diagnostic routine setting, no additional control samples were included in the present study.

Baseline serum total tryptase

Baseline tryptase was measured in all 58 patients. Tryptase was measured prior to the *KIT* D816V mutation analysis of the screening blood sample in 11 patients (range 15-721 days, median 78 days), at the same day as the *KIT* D816V analysis in 46 patients and later than the *KIT* D816V analysis in one patient (593 days). Tryptase measurements were performed using the ImmunoCAP Tryptase assay (Thermo Fisher Scientific, Uppsala, Sweden).

BM investigation

The BM investigation included morphologic evaluation of blood smear and morphologic evaluation of BM aspiration smear, touch imprint, aspiration clot and trephine biopsy in all patients. The BM investigation also included CD2, CD25, CD34, CD117 and MC tryptase immunohistochemical analysis of the BM aspiration clot and/or biopsy. In all patients, flow cytometric analysis of BM aspirate was also performed using antibodies for the following antigens; CD2, CD25, CD33, CD34, CD45, CD117, as previously described (23).

Statistical analysis

Linear regression analysis, correlation analysis, paired t-test and Mann–Whitney U-test were performed using IBM SPSS Statistics 21 software (IBM Corporation, Armonk, NY, USA). A P-value <0.05 was considered statistically significant.

Results

Screening blood samples

Screening blood samples were obtained and analyzed for the *KIT* D816V mutation at a median of 87 days (range 9 – 676 days) before the BM investigation was performed. In 23 of the 58 patients, a second blood sample was also obtained before the time of the BM biopsy.

Accepted Article

These second blood samples always produced the same D816V-positive or D816V-negative result as the first samples, except for one patient where the first blood sample tested D816V-positive and the second sample tested D816V-negative (the patient ended up with SM diagnosis).

A total of 44 of the 58 blood screening samples tested positive for the *KIT* D816V mutation. In 43 of 44 patients (98%) who tested positive in the screening sample, a diagnosis of SM was subsequently established in the BM investigation. In the single patient with a D816V-positive screening sample that did not fulfil SM criteria, two follow-up blood samples and the BM aspirate also tested D816V-positive, thus demonstrating that the D816V-positive status of the patient was highly reliable. In the flow cytometric analysis of BM aspirate, a small population of CD25-positive MCs was detected at a frequency below the standard detection limit of 0.002%. The patient presented with hymenoptera venom anaphylaxis, a tryptase level of 6.2 ng/mL and no skin lesions. According to the MC activation syndrome consensus proposal by Valent *et al.* (2012), this patient should formally be classified as “monoclonal MC activation syndrome” (24). This condition has been proposed to represent “sub-diagnostic SM” or “low-burden SM” (13).

A total of 14 of the 58 screening blood samples tested D816V-negative. Of these 14 patients, five were diagnosed with SM in the BM investigation. These five patients all tested D816V-positive in BM aspirate (two only in mRNA-based analysis as previously described, (25)). One of the five patients presented with skin lesions and the remaining four presented with anaphylaxis (hymenoptera venom-induced in three of four). None of the remaining nine patients who tested D816V-negative in the screening blood sample fulfilled any of the WHO diagnostic criteria of SM (apart from tryptase >20 ng/mL in two patients). None of these nine patients had mastocytosis skin lesions and a diagnosis of mastocytosis was thereby excluded in all these patients. Also, no hematologic diagnosis, or any other diagnosis, was established

in the BM investigation in any of these nine patients. See **Supplementary Table S1** for analysis results of each patient.

SM patients

Of the 58 included patients, a total of 48 patients thereby fulfilled criteria of SM. Forty three of 48 SM patients (90%) tested D816V-positive in the screening blood sample (**Table 1**). SM patients were classified as ISM with skin lesions (N=26; this group includes all patients that presented with skin lesions) and BM mastocytosis (BMM) (N=22). Nineteen of 48 SM patients (40%) fulfilled the WHO major criterion of MC aggregates. All SM patients fulfilled the WHO minor criteria of MC CD2 and/or CD25 expression and atypical MC morphology. Of the 26 ISM patients with skin lesions (skin lesions were biopsy verified in 24 patients), 12 fulfilled the major criterion (46%) and 25 tested D816V-positive in the screening blood sample (96%). Of the 22 BMM patients, 7 fulfilled the major criterion (32%), and 18 tested D816V-positive in the screening blood sample (82%). Four out of five “false negative” screening results thereby occurred in SM patients without skin lesions (**Table 1**). The false negative rate of 18% in BMM patients also demonstrates the extent to which the diagnostic value is limited in these patients and underlines that a BM investigation is mandatory in all adult patients with clear signs of, or highly suspected SM, regardless of the *KIT* D816V mutation status.

KIT D816V allele burden

The mutation level in the 44 screening blood samples that tested *KIT* D816V-positive ranged from 0.001 to 2.2% D816V-positive alleles (median = 0.03%). In 40 of these patients, the D816V allele burden was also quantified in blood at the time of the BM investigation. The median fold change, calculated as the D816V allele burden in the blood sample from the time

of the BM investigation relative to the D816V allele burden in the screening blood sample, was 1.0 (range 0.13 – 7), which demonstrates no tendency to a change in allele burden over time (**Figure 1**). Linear regression analysis with fold change as a function of days between the two blood samples was non-significant ($P=0.23$), and no significant difference was detected between allele burden in the two paired blood samples when analyzed using paired t-test ($P=0.38$). Altogether, this demonstrates highly stable mutation levels in blood over time.

The D816V allele burden was compared in SM patients with positive screening blood samples, with and without the major criterion fulfilled (**Figure 2A**). There was a tendency towards a higher allele burden in patients fulfilling the major criterion, although non-significant (median allele burden 0.02% without major vs. 0.07% with major; Mann–Whitney U-test: $P=0.08$).

The D816V allele burden was also compared in SM patients with positive blood screening samples, with and without skin lesions (**Figure 2B**). The allele burden was significantly higher in patients with skin lesions (median allele burden 0.009% without skin lesions vs. 0.09% with skin lesions; Mann–Whitney U-test: $P<0.001$).

There was a highly significant correlation between the D816V allele burden in BM aspirate and blood samples from the time of the BM investigation in patients who tested positive in both samples ($P<0.001$). No significant difference was detected between the D816V allele burden in BM aspirate and blood samples from the time of the BM investigation in patients who tested positive in both samples (BM median allele burden 0.04% vs. blood median allele burden 0.02%; paired t-test: $P=0.17$).

Baseline serum total tryptase

Tryptase was measured in all patients included in the study. Fifteen of the 48 SM patients (31%) fulfilled the WHO minor criterion of total serum tryptase levels >20 ng/mL (**Figure 3**). In ISM patients with skin lesions, 10 of 26 patients (38%) had tryptase levels <11.4 ng/mL and 17 of 26 patients (65%) had tryptase levels ≤ 20 ng/mL. In BMM patients, 12 of 22 patients (55%) had tryptase levels <11.4 ng/mL and 16 of 22 patients (73%) had tryptase levels ≤ 20 ng/mL. All 12 BMM patients with tryptase levels <11.4 ng/mL and 15 of 16 patients with tryptase levels ≤ 20 ng/mL tested D816V-positive in the screening blood sample, thus demonstrating the potential of the mutation analysis for identifying SM in patients with low tryptase levels and no skin lesions.

There was a significant correlation between tryptase levels and the D816V allele burden in screening blood samples in patients who tested positive ($P=0.005$). No significant difference in tryptase levels in patients with BMM vs. ISM with skin lesions was detected (median 9.2 ng/mL in BMM vs. 14.3 ng/mL in ISM with skin lesions; Mann–Whitney U-test: $P=0.12$).

Of the 22 BMM patients, the WHO major criterion was absent in 15 patients. Of these, tryptase levels were ≤ 20 ng/mL in 13 patients and <11.4 ng/mL in 11 patients. Most of the BMM patients thereby only had very discrete BM infiltration and were diagnosed with SM based on three WHO minor criteria only (*KIT* mutation, MC CD2 and/or CD25 expression and atypical MC morphology).

Twelve BMM patients had tryptase levels <11.4 ng/mL and all presented with anaphylaxis (hymenoptera venom-induced in 11 patients), which thereby characterize this diagnostically challenging group of patients. All these 12 patients tested D816V-positive in the screening blood sample. See **Supplementary Table S1** for analysis results of each patient.

Discussion

Sensitive *KIT* D816V mutation analysis of blood has been proposed as a tool to guide BM investigation in suspected SM (20, 21). We here report the first prospective study in which the result of sensitive *KIT* D816V mutation analysis of the screening blood sample used to guide BM biopsy is compared to the final outcome of the subsequent BM investigation. With 43 of 44 patients with a D816V-positive screening blood sample ending up with a final diagnosis of SM in the BM investigation, the study demonstrates a D816V-positive blood sample as a reliable predictor of SM. The single *KIT* D816V-positive patient in whom a diagnosis of SM was not established was diagnosed with monoclonal MC activation syndrome (24). No technical false positive results were thereby observed in any patient in the present study, thus demonstrating the genetics-based approach in suspected SM to be highly specific.

Only approximately one third of SM patients fulfilled the WHO minor criterion of a tryptase level >20 ng/mL and almost half of the SM patients had tryptase levels within the normal range. With almost all SM patients with low tryptase levels testing D816V-positive in the screening blood sample, including those without skin lesions, this study demonstrates a high value of implementing sensitive *KIT* D816V mutation analysis in the diagnostic algorithm for suspected SM.

We have previously demonstrated that the vast majority (94%) of already diagnosed SM patients test *KIT* D816V mutation positive in blood when using a sufficiently sensitive method (14). However, in line with similar studies from other groups also detecting the *KIT* D816V mutation in blood in high numbers of SM patients, (19, 26, 27), our previous study was primarily based on blood samples obtained at, or later than, the time of the BM investigation. These blood samples were not used in the diagnostic work-up to guide the BM biopsy, but were instead sampled after the decision to perform a BM biopsy was made (at the

Accepted Article

day of the BM biopsy or after the SM diagnosis was established). These previous studies were therefore unable to provide data on the diagnostic outcome of including *KIT* D816V mutation analysis of blood samples in the diagnostic work-up in suspected SM. However, we do demonstrate that mutation levels in screening blood samples are highly similar to mutation levels in blood at the time of the BM investigation. Laboratory studies on these later blood samples typically biobanked in many mastocytosis centers are therefore likely to be highly representative of the screening samples in ISM patients, in spite of the different clinical context.

In the present study, the *KIT* D816V allele burden was not significantly higher in SM patients who fulfilled the major criterion compared to patients who did not. This result is in line with a previous result by Hoermann *et al.* (2014) who observed no significant correlation between the *KIT* D816V allele burden and the percentage of MCs in the BM biopsy or BM smear (27). Together, these results indicate that the association between the allele burden and degree of MC infiltration in the BM is limited, likely because the allele burden also reflects the degree of non-MC lineage involvement in addition to the neoplastic MC burden (19). However, the lack of statistical significance in these studies may also be due to lack of statistical power as a result of insufficient numbers of patients.

In the present study, 90% of SM patients tested D816V-positive in the screening blood sample. This number is slightly lower than the 94% of SM patients with D816V-positive blood samples detected in our previous study with already diagnosed patients (14). This difference is most likely explained by the higher number of ISM patients without skin lesions (BMM) in the present study. Only 82% of BMM patients tested D816V-positive in the screening blood sample and the D816V allele burden in blood was lower compared to ISM patients with skin lesions. This result is in line with a previous study detecting *KIT* mutation in blood with the lowest frequency in BMM patients compared to other SM subtypes (19).

Accepted Article

These results underline the need for performing the mutation analysis with the highest possible analytical sensitivity (median D816V allele burden in screening blood samples was 0.03%), in particular because the test is of greatest value in suspected BMM where mutation levels are lowest.

We are not able to provide a true estimate of the statistical sensitivity (true positive rate) of the approach in the present study. This would require a full BM investigation of all patients with suspected SM where blood was screened for the *KIT* D816V mutation. In our experience, only a minor fraction of patients testing negative for the D816V mutation subsequently undergo a full BM investigation and the study would be difficult to justify ethically because of the relatively low number of *a priori* expected additional SM patients being diagnosed. However, five patients were diagnosed with D816V-positive SM although they tested D816V-negative in the screening blood sample in the present study, thus demonstrating that a D816V-negative result should not exclude a BM investigation if clearly indicated by other markers or the clinical picture, in particular in patients with venom anaphylaxis and elevated tryptase which characterize these patients in the present study. Recently, it has also been demonstrated that a high fraction of patients with hymenoptera venom-induced anaphylaxis with hypotension, absence of mastocytosis skin lesions and normal tryptase levels fulfil criteria of D816V-positive SM (15). In line with the present study, this previous study also identifies significant numbers of patients with BMM that due to the lack of skin lesions and normal tryptase levels often would not qualify for a BM investigation. Collectively, these results demonstrate that SM may be much more prevalent among patients with hymenoptera venom-induced anaphylaxis than previously expected. Additional studies on this patient group as well as a more widespread use of sensitive *KIT* D816V mutation analysis in clinical practice will further substantiate this observation. Ideally, these additional studies should include a systematic application of all available

diagnostic tests for SM and a full BM investigation, including sensitive flow cytometric analysis of BM aspirate, in all patients.

In conclusion, we demonstrate that a D816V-positive result in a screening blood sample allows identification of significant numbers of SM patients with no skin lesions and normal tryptase levels. In particular, the test identifies SM among patient with hymenoptera venom-induced anaphylaxis in whom the diagnosis would otherwise most probably have been missed, with potential severe implications. This prospective study thereby provides strong experimental evidence to support the inclusion of *KIT* D816V mutation analysis in the diagnostic algorithms for suspected SM in adults, in particular in patients with hymenoptera venom-induced anaphylaxis. However, the observed false negative screening results also underline that BM investigation is mandatory in all adult patients with clear signs of, or highly suspected SM, regardless of the *KIT* mutation status.

Acknowledgments

None

Author Contributions

TK and SBO conceived the study idea and developed it in collaboration with MBM, HV, CBJ, CGM, HFK and MO. TK was responsible for interpretation of *KIT* D816V mutation analysis, performed statistical analyses, wrote the initial draft and organized the writing.

MBM was responsible for analysis and interpretation of pathology and flow cytometry data. SBO, HV, CBJ, CGM and HFK evaluated the patients clinically. All authors participated in the discussion and interpretation of the results. All authors critically revised the manuscript for intellectual content and approved the final version before submission.

Disclosure of Conflicts of Interest

The authors have no conflicts of interest to declare.

Tables

Table 1. SM patients grouped according to the presence of skin lesions, presence of WHO major criterion and *KIT* D816V mutation status of the screening blood sample.

	Systemic mastocytosis (N = 48)			
	Bone marrow mastocytosis (N = 22)		ISM with skin lesions (N = 26)	
	Major criterion not fulfilled (N = 15)	Major criterion fulfilled (N = 7)	Major criterion not fulfilled (N = 14)	Major criterion fulfilled (N = 12)
D816V-negative screening blood sample	1 of 15 (7%)	3 of 7 (43%)	1 of 14 (7%)	0 of 12 (0%)
D816V-positive screening blood sample	14 of 15 (93%)	4 of 7 (57%)	13 of 14 (93%)	12 of 12 (100%)

Figures

Figure 1. Change in *KIT* D816V allele burden over time, calculated as the D816V allele burden in the blood sample from the time of the BM investigation relative to the D816V allele burden in the screening blood sample (N=40). A fold change = 1 thus corresponds to no change in the D816V allele burden over time and the values of 0.1 and 10 correspond to 10-fold decreases and increases, respectively. The line represents the result of linear regression analysis.

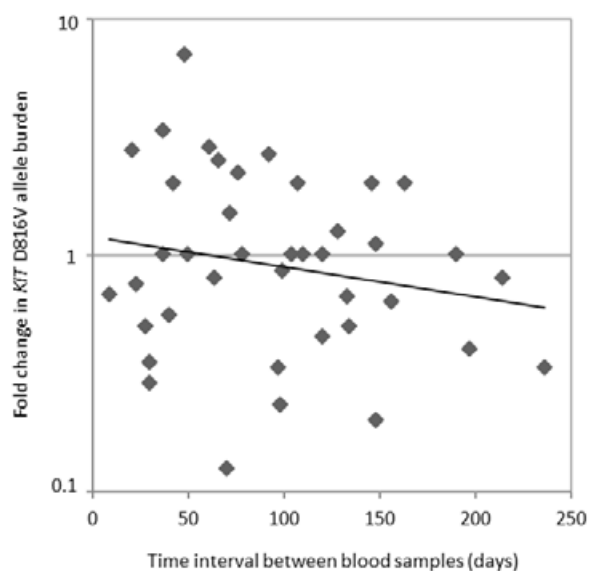


Figure 2. The *KIT* D816V allele burden in [A] SM patients with and without the WHO major criterion fulfilled, and [B] BMM patients and ISM patients with skin lesions. Only SM patients with positive screening blood samples are included in this figure (total N=43).

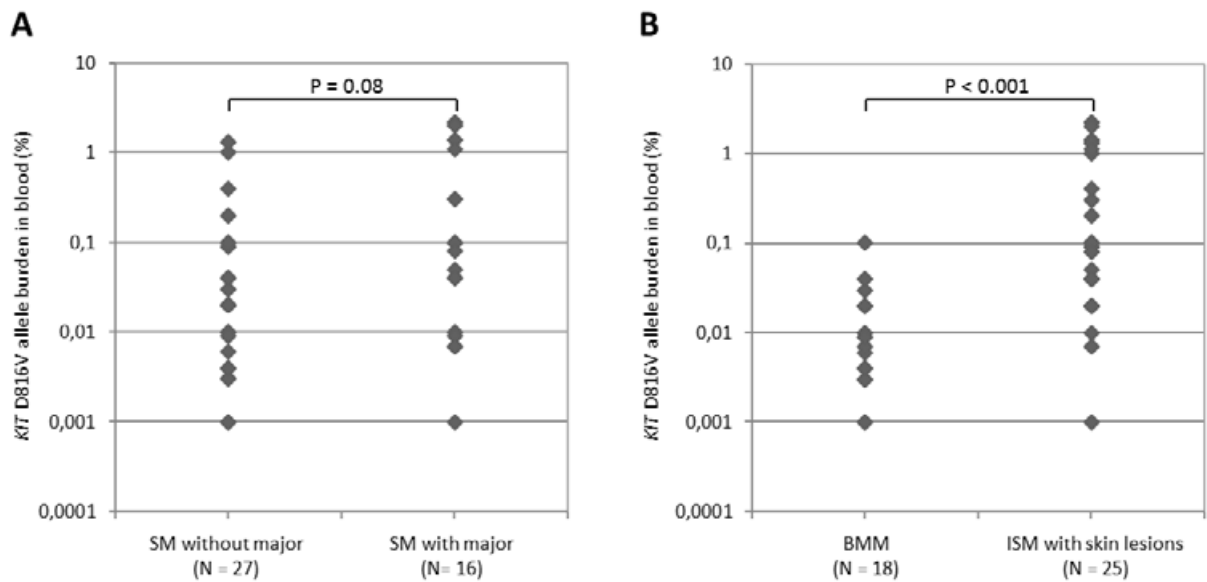
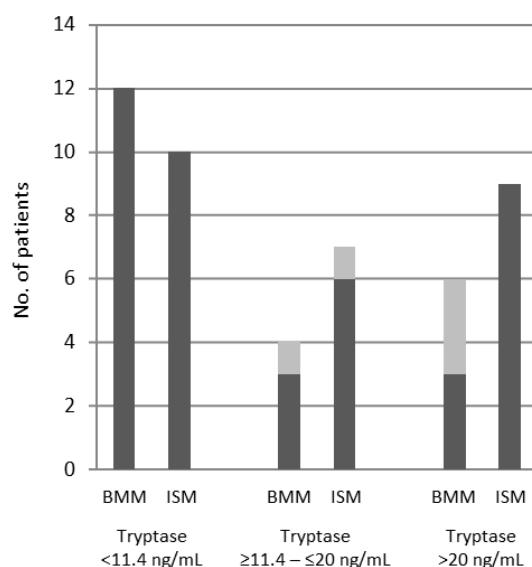


Figure 3. Distribution of serum tryptase levels in BMM (N=22) and ISM patients with skin lesions (N=26). Dark grey bars represent patients with *KIT* D816V-positive screening blood samples (N=43) and light grey bars represent patients with *KIT* D816V-negative screening blood samples (N=5).



References

- (1) Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Conference Report of 'Year 2000 Working Conference on Mastocytosis'. *Leuk Res* 2001;**25**:603–625.
- (2) Horny HP, Metcalfe DD, Bennett JM, Bain BJ, Akin C, Escribano E, Valent P. Mastocytosis. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al., editors. *World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th ed. Lyon, France: International Agency for Research and Cancer (IARC) Press; 2008. pp 54–63.
- (3) Valent P, Horny HP, Li CY, Longley JB, Metcalfe DD, Parwaresch RM et al. Mastocytosis (Mast cell disease). In: Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. *World Health Organization (WHO) Classification of Tumours. Pathology & Genetics. Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: International Agency for Research and Cancer (IARC) Press; 2001. pp 291–302.

- (4) Valent P, Akin C, Escribano L, Fodinger M, Hartmann K, Brockow K, et al. Standards and standardization in mastocytosis: Consensus statements on diagnostics, treatment recommendations and response criteria. *Eur J Clin Invest* 2007;**37**:435-453.
- (5) Pieri L, Bonadonna P, Elena C, Papayannidis C, Grifoni FI, Rondoni M, et al. Clinical presentation and management practice of systemic mastocytosis. A survey on 460 Italian patients. *Am J Hematol* 2016;**91**:692-699.
- (6) Reichard KK, Chen D, Pardananani A, McClure RF, Howard MT, Kurtin PJ, et al. Morphologically occult systemic mastocytosis in bone marrow: clinicopathologic features and an algorithmic approach to diagnosis. *Am J Clin Pathol* 2015;**144**:493-502.
- (7) Theoharides TC, Valent P, Akin C. Mast Cells, Mastocytosis, and Related Disorders. *N Engl J Med* 2015;**373**:163-172.
- (8) Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. *Allergy* 2008;**63**:226-232.
- (9) Broesby-Olsen S, Farkas DK, Vestergaard H, Hermann AP, Moller MB, Mortz CG, et al. Risk of solid cancer, cardiovascular disease, anaphylaxis, osteoporosis and fractures in patients with systemic mastocytosis: A nationwide population-based study. *Am J Hematol* 2016;**91**:1069-1075.
- (10) Oude Elberink JN, de Monchy JG, Kors JW, van Doormaal JJ, Dubois AE. Fatal anaphylaxis after a yellow jacket sting, despite venom immunotherapy, in two patients with mastocytosis. *J Allergy Clin Immunol* 1997;**99**:153-154.
- (11) van der Veer E, van der Goot W, de Monchy JG, Kluin-Nelemans HC, van Doormaal JJ. High prevalence of fractures and osteoporosis in patients with indolent systemic mastocytosis. *Allergy* 2012;**67**:431-438.
- (12) Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin North Am* 2006;**26**:451-463.
- (13) Pardananani A, Chen D, Abdelrahman RA, Reichard KK, Zblewski D, Wood AJ, et al. Clonal mast cell disease not meeting WHO criteria for diagnosis of mastocytosis: clinicopathologic features and comparison with indolent mastocytosis. *Leukemia* 2013;**27**:2091-2094.
- (14) Kristensen T, Vestergaard H, Bindslev-Jensen C, Moller MB, Broesby-Olsen S. Sensitive KIT D816V mutation analysis of blood as a diagnostic test in mastocytosis. *Am J Hematol* 2014;**89**:493-498.
- (15) Zanotti R, Lombardo C, Passalacqua G, Caimmi C, Bonifacio M, De MG, et al. Clonal mast cell disorders in patients with severe Hymenoptera venom allergy and normal serum tryptase levels. *J Allergy Clin Immunol* 2015;**136**:135-139.
- (16) Garcia-Montero AC, Jara-Acevedo M, Teodosio C, Sanchez ML, Nunez R, Prados A, et al. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages

in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Blood* 2006;**108**:2366-2372.

- (17) Nagata H, Worobec AS, Oh CK, Chowdhury BA, Tannenbaum S, Suzuki Y, et al. Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc Natl Acad Sci U S A* 1995;**92**:10560-10564.
- (18) Longley BJ, Tyrrell L, Lu SZ, Ma YS, Langley K, Ding TG, et al. Somatic c-KIT activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. *Nat Genet* 1996;**12**:312-314.
- (19) Jara-Acevedo M, Teodosio C, Sanchez-Munoz L, Alvarez-Twose I, Mayado A, Caldas C, et al. Detection of the KIT D816V mutation in peripheral blood of systemic mastocytosis: diagnostic implications. *Mod Pathol* 2015;**28**:1138-1149.
- (20) Arock M, Sotlar K, Akin C, Broesby-Olsen S, Hoermann G, Escribano L, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. *Leukemia* 2015;**29**:1223-1232.
- (21) Valent P, Escribano L, Broesby-Olsen S, Hartmann K, Grattan C, Brockow K, et al. Proposed diagnostic algorithm for patients with suspected mastocytosis: a proposal of the European Competence Network on Mastocytosis. *Allergy* 2014;**69**:1267-1274.
- (22) Broesby-Olsen S, Oropeza AR, Bindslev-Jensen C, Vestergaard H, Moller MB, Siebenhaar F, et al. Recognizing mastocytosis in patients with anaphylaxis: value of KIT D816V mutation analysis of peripheral blood. *J Allergy Clin Immunol* 2015;**135**:262-264.
- (23) Kristensen T, Vestergaard H, Moller MB. Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. *J Mol Diagn* 2011;**13**:180-188.
- (24) Valent P, Akin C, Arock M, Brockow K, Butterfield JH, Carter MC, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *Int Arch Allergy Immunol* 2012;**157**:215-225.
- (25) Kristensen T, Broesby-Olsen S, Vestergaard H, Bindslev-Jensen C, Moller MB. Comparison of gDNA-based versus mRNA-based KIT D816V mutation analysis reveals large differences between blood and bone marrow in systemic mastocytosis. *Br J Haematol* 2017; *In Press*.
- (26) Erben P, Schwaab J, Metzgeroth G, Horny HP, Jawhar M, Sotlar K, et al. The KIT D816V expressed allele burden for diagnosis and disease monitoring of systemic mastocytosis. *Ann Hematol* 2014;**93**:81-88.
- (27) Hoermann G, Gleixner KV, Dinu GE, Kundi M, Greiner G, Wimazal F, et al. The KIT D816V allele burden predicts survival in patients with mastocytosis and correlates with the WHO type of the disease. *Allergy* 2014;**69**:810-813.