

KIT D816V mutation burden does not correlate to clinical manifestations of indolent systemic mastocytosis

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Background: Clinical manifestations of indolent systemic mastocytosis (ISM) comprise mediator-related symptoms, anaphylaxis, and osteoporosis. A new sensitive method for *KIT* D816V mutation detection allows quantification of the level of mutation-positive cells.

Objective: To investigate whether the fraction of *KIT* D816V positive cells in peripheral blood (PB) or bone marrow (BM) aspirate in adult patients with ISM correlates with clinical manifestations of the disease.

Methods: We included 48 adult patients with ISM (28 females/20 males) from our center in whom the *KIT* D816V mutation level in both BM aspirate and PB was analyzed. For each patient, the severity of mediator-related symptoms (skin, gastrointestinal, musculoskeletal, and neuropsychiatric) and episodes of anaphylaxis were evaluated by interview and medical record files. Bone mineral density was determined by using dual-energy x-ray absorptiometry.

Results: Median fraction (range) of *KIT* D816V positive cells was 0.6 (0.01%-90%) in BM and 0.3 (0.003%-49%) in PB. Mutation level did not differ between patients with none/mild symptoms and patients with moderate/severe symptoms, patients with and without anaphylaxis, or patients with osteoporosis/osteopenia and normal bone mineral density. No significant differences in clinical profile were detected in patients with different levels of mutation except for an indication of longer disease duration and age in patients with highest mutation levels.

Conclusion: To our knowledge, this is the first report on the clinical impact of the fraction of *KIT* D816V mutation positive cells in ISM, which in the present study does not seem to correlate with clinical manifestations of the disease. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Indolent systemic mastocytosis, *KIT* D816V, mutation burden, allele burden, mast cell, anaphylaxis, osteoporosis

Systemic mastocytosis (SM) is a heterogeneous group of diseases with clonal expansion of mast cells (MCs) in various

Abbreviations used

BM: Bone marrow
BMD: Bone mineral density
DXA: Dual-energy x-ray absorptiometry
GI: Gastrointestinal
ISM: Indolent systemic mastocytosis
MCs: Mast cells
PB: Peripheral blood
SM: Systemic mastocytosis

organs.¹ Almost all adult patients with SM carry the somatic *KIT* A2447T mutation leading to D816V amino acid substitution, resulting in autoactivation of the *KIT* receptor tyrosine kinase and ligand-independent MC proliferation and differentiation.²⁻⁴ The World Health Organization classifies SM into distinct categories, of which indolent SM (ISM) is by far the most frequent.^{5,6} Even though ISM is characterized by a persistent, nonaggressive course and a normal life expectancy,^{7,8} symptoms related to MC mediator release (skin symptoms, gastrointestinal [GI] symptoms, musculoskeletal pain and neuropsychiatric symptoms, and even life-threatening anaphylaxis^{9,10}) can be debilitating. Furthermore, an increased frequency of osteoporosis even at young age is seen in ISM.^{11,12} The precise pathogenesis and explanation for the varying severity of symptoms in ISM is not clear.

We recently introduced the fraction of mutation-positive cells in both peripheral blood (PB) and bone marrow (BM) aspirate as new variables generally applicable in patients with SM by using a sensitive and quantitative *KIT* D816V mutation analysis.¹³ In addition to detecting the mutation in BM, the method consistently allows detection of the mutation in PB in ISM.¹⁴ In the present study, we tested whether the fraction of *KIT* D816V mutation-positive cells in PB and BM was different in groups of adult patients with ISM with different severity of MC mediator release symptoms, occurrence of anaphylaxis, and osteoporosis.

METHODS

Patients

From our cohort of adult patients diagnosed with ISM at the Mastocytosis Centre, Odense University Hospital, Denmark, we included 48 patients in this study in whom the *KIT* D816V mutation level in both BM aspirate and PB was determined by using real-time quantitative PCR. The following patients were excluded: *KIT* D816V mutation-negative patients ($n = 1$), patients in whom the mutation level was not available or performed only in BM, PB, or skin ($n = 14$), and patients who had received cytoreductive treatment at other hospitals prior to determination of the mutation level at our center ($n = 3$).

The ISM diagnosis was based on adequate BM biopsy specimens in all patients in accordance with the 2008 World Health Organization diagnostic criteria⁵ and by exclusion of aggressive subtypes of SM. Onset of disease was defined as the time of onset of skin elements or as the first severe MC mediator

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release episode, whatever came first. Time of diagnosis was defined as time of positive BM biopsy.

Antimediator therapy in all patients was recorded by medical record files. Local ethics guidelines were followed for this study.

Mutation analysis

Sensitive and quantitative *KIT* D816V analysis using mutation-specific real-time PCR was performed by using DNA extracted from unfractionated PB and BM aspirate as previously described.¹³

Results are calculated as the fraction of *KIT* D816V mutation-positive heterozygous cells (hereafter “fraction of *KIT* D816V positive cells” or “mutation level”) as previously described in detail,¹³ which is in line with the general assumption that most cells carry the mutation heterozygously.¹⁵

As part of the data-analysis, we stratified patients by mutation level in PB into 4 subgroups to look for differences in the clinical parameters investigated. To allow evaluation of patients with the highest levels of mutation and separately and create groups of approximately similar sizes, we chose the following cutoffs for the subgroups: (1) 0.003% to 0.1%, (2) 0.1% to 1%, (3) 1% to 10%, and (4) 10% to 100% mutation-positive cells.

Mediator-related symptoms

All 48 included patients had been clinically examined by one of the authors (S.B.-O.) and on the basis of this and existing medical record files, the mediator-related symptoms were graded 0 = none (no symptoms), 1 = mild (mild or infrequent symptoms not requiring therapy or controlled by on-demand therapy), 2 = moderate (moderate symptoms that require and can be controlled by daily therapy), and 3 = severe (severe symptoms that in spite of daily therapy are not well controlled) in accordance with previous reports.^{6,16} Symptoms were evaluated for 4 parameters: skin (pruritus, whealing, or flushing), GI (diarrhea, abdominal pain/cramping, or gastroesophageal reflux), musculoskeletal (bone pain or myoarthralgia), and neuropsychiatric (depression, loss of concentration, moods, or fatigue). Skin symptom score was thus not area of skin involvement (skin lesions) but severity of mediator-related skin symptoms.

For comparative analysis with respect to mutation levels, patients were divided into 2 groups: one showing no to mild symptoms, and the other with moderate to severe symptoms.

Anaphylaxis

Anaphylaxis was defined according to international guidelines.¹⁷ Mild episodes that by some definitions of anaphylaxis¹⁸ are graded as level 1 were excluded. Time point of retrospective evaluation in each patient was defined as the time of mutation analysis. Anaphylactic episodes that were not confirmed by hospitalization or medical record files were recorded on the basis of patient history by careful evaluation and exclusion of non-mastocytosis-related events.

Bone mineral density

Areal bone mineral density (BMD) of the lumbar spine (L1-L4) and nondominant total hip region was measured by using dual-energy x-ray absorptiometry (Hologic Discovery, Waltham, Mass). *T* and *Z* scores were calculated by using a Hologic-provided reference range at the spine and the National Health and Nutrition Examination Survey III reference range at the hip.¹⁹ In our unit, the coefficient of variation for measurements of total hip and spine is 1.5% at each site. *T* score is the number of SDs below the young adult mean in the same sex, and *Z* score is the age-matched equivalent. In none of the patients was the *T* score or the *Z* score of the hip lower than that of the spine; thus, values only for the spine were chosen for analysis in this study. Osteoporosis was defined according to World Health Organization guidelines as a *T*-score equivalent to or less than -2.5 and osteopenia as a *T* score between -1.0 and -2.5 . If more than 1 scan had been performed, results from the dual-energy x-ray absorptiometry scan closest to SM diagnosis were used. In 5 patients who were on antiosteoporotic treatment prior to the

determination of mutation level, the result of first dual-energy x-ray absorptiometry scan was used for analysis based on the assumption that the antiosteoporotic treatment should not affect the mutation level.

Statistical analysis

Data are presented as median (range) for continuous variables and as frequencies (%) for categorical variables. Box plots are depicted as classic 5 number summaries: minimum, lower quartile, median, upper quartile, and maximum. Between-group differences were assessed with the Mann-Whitney *U* or Kruskal Wallis test for continuous variables and the χ^2 or Fisher exact test for categorical variables. Associations between continuous variables were analyzed by correlation analysis (Spearman correlation coefficients ρ). Reported *P* values are considered statistically significant if below .05. Statistics were performed by using SPSS 20.0 (IBM Corporation, Armonk, NY).

RESULTS

Patient demographics and clinical characteristics

Findings are summarized in Table I. The slight female preponderance, median age at onset, and disease duration was similar to reports on SM by others^{9,10,12} as was the median level of baseline serum tryptase (Thermo Fisher, Uppsala, Sweden), which was 30.1 (5.4-294) ng/mL.

All patients except 1 had classic urticaria pigmentosa lesions in the skin, ranging from fewer than 10 elements up to widespread skin involvement. Skin lesions were the presenting symptom in more than 90% of the patients. None of the patients had teleangiectasia macularis eruptiva perstans, but several patients had dilated, sometimes confluent, small blood vessels in conjunction with the urticaria pigmentosa lesions especially with long disease duration and probably secondary to histamine load in the skin. Interestingly, when dividing patients by skin area involvement into (1) *none to mild* ($n = 19$) and (2) *moderate to severe* ($n = 29$) on the basis of clinical examination and photographs, we found no significant differences when comparing the median mutation level in these 2 groups (PB: $P = .19$; BM: $P = .34$).

On evaluating the use of antimediator therapy, we found that 19 (39.6%) patients had received therapy before referral—mainly nonsedating H1 antihistamines in low dose—and often on-demand therapy. After referral/diagnosis, 37 (77.1%) patients received antimediator therapy (H1 antihistamines, H2 antihistamines, montelukast, cromolyn sodium—often combined). These frequencies of therapy before and after referral are similar to reports by others.¹²

KIT D816V

The median fraction of *KIT* D816V mutation-positive cells was 0.6 (0.01%-90%) in BM and 0.3 (0.003%-49%) in PB. As expected, and in line with previous findings,¹⁴ a highly significant correlation between mutation burden in BM and PB was detected ($r = 0.9$, $P < .001$), illustrated in Fig 1. We found no difference in the mutation level in men and women (PB: $P = .85$, BM: $P = .96$).

Findings of stratification of patients by mutation level into 4 subgroups are summarized in Table I. No significant differences in clinical profile in these subgroups were observed concerning mediator-related symptoms, anaphylaxis, and osteoporosis/osteopenia ($P > .05$). Median BMD *Z* score was higher in the subgroup with the highest mutation level; however, ranges were clearly overlapping and the difference not significant ($P > .05$). The

TABLE I. Demographic and clinical data in total study population and in 4 subgroups divided by KIT D816V mutation level

Variable	Total (N = 48)	Mutation level			
		0.003%-0.1% (n = 16)	0.1%-1% (n = 14)	1%-10% (n = 11)	10%-100% (n = 7)
Males	20 (41.7)	7 (43.8)	5 (35.7)	6 (54.5)	2 (28.6)
Age at onset* (y)	36 (2-64)	24 (11-44)	36 (20-62)	38 (2-64)	46 (20-58)
Age at diagnosis† (y)	45 (19-77)	43 (19-49)	46 (27-65)	62 (31-77)	59 (40-77)
Disease duration at diagnosis (y)	11 (1-41)	8 (1-29)	5 (1-25)	18 (1-41)	19 (5-25)
Moderate-severe skin symptoms‡	16 (33.3)	7 (43.8)	2 (14.3)	3 (27.3)	4 (57.1)
Moderate-severe GI symptoms§	15 (31.3)	7 (43.8)	2 (14.3)	3 (27.3)	3 (42.9)
Moderate-severe musculoskeletal symptoms	9 (18.8)	5 (31.3)	2 (14.3)	1 (9.1)	1 (14.3)
Moderate-severe neuropsychiatric symptoms¶	8 (16.7)	3 (18.8)	2 (14.3)	1 (9.1)	2 (28.6)
Anaphylaxis	17 (35.4)	8 (50.0)	4 (28.6)	3 (27.3)	2 (28.6)
Osteoporosis or osteopenia	32 (66.7)	9 (56.3)	10 (71.4)	8 (72.7)	4 (57.1)
BMD Z score (SD)	-1 (-3.7 to +2.0)	-1.3 (-3.7 to +2.2)	-0.9 (-3.0 to +1.2)	-1.1 (-2.9 to +1.1)	+0.1 (-3.7 to +2.2)

Note. Results are expressed as number of patients (percentage of total patients studied) for categorical variables and median (range) for continuous variables.

*Date of appearance of skin lesions or first severe MC mediator release episode.

†Date of positive BM biopsy.

‡Pruritus, whealing, flushing.

§Diarrhea, abdominal pain/cramping, reflux.

||Bone pain, myoarthralgia.

¶Depression, loss of concentration, moods, fatigue.

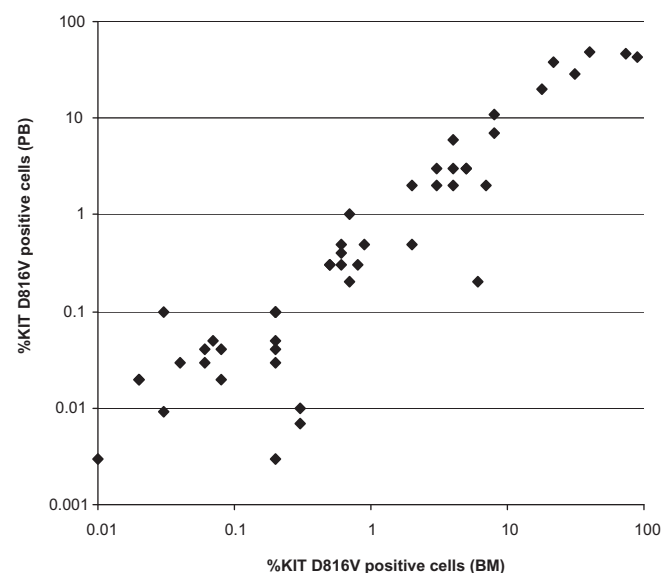


FIG 1. Mutation burden in BM and PB in the 48 patients studied demonstrating a highly significant mutual correlation ($r = 0.9$, $P < .001$). Median fraction of *KIT* D816V positive cells was 0.6 (0.01%-90%) in BM and 0.3 (0.003%-49%) in PB.

median age at onset, age at diagnosis, and disease duration were higher in the subgroups with the highest mutation level—however not significantly ($P > .05$)—and on analyzing the whole group, no clear significant correlation between the fraction of mutation-positive cells in PB or BM and disease duration at the time of diagnosis was detected (Fig 2) (PB: $r = 0.2$, $P = .12$; BM: $r = 0.04$, $P = .16$).

Of note, among the 7 patients with a high mutation level above 10% we have observed no signs of progression to aggressive disease in a follow-up period of 18 months (7-38).

The patient with the highest mutation level had 90% mutation-positive cells in BM. This patient was a 59-year-old woman with ISM with moderate-severe skin symptoms, but otherwise very

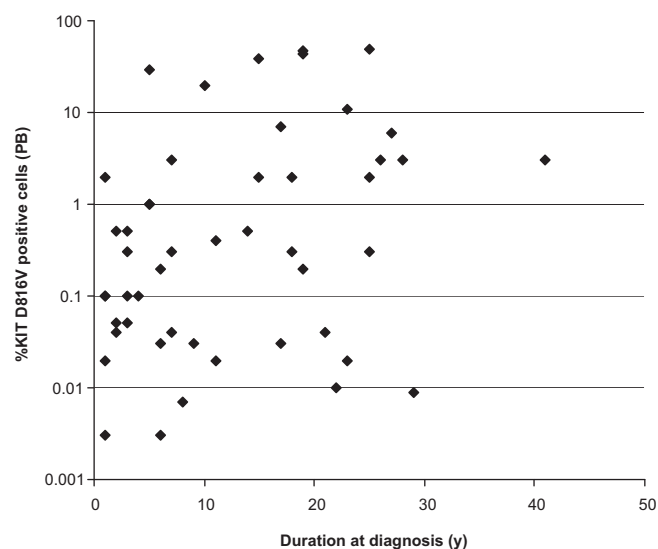


FIG 2. Mutation burden in PB in relation to duration of disease at diagnosis. No significant correlation is seen ($r = 0.05$, $P = .13$). A similar result was found for BM ($r = 0.04$, $P = .16$).

few and mild symptoms, normal Z score, and no evidence of hematologic abnormalities.

Mediator-related symptoms

No significant difference in the fraction of *KIT* D816V mutation-positive cells was detected between the groups with different severity of the 4 included types of MC mediator-related symptoms (skin, GI, musculoskeletal, and neuropsychiatric).

Skin symptoms consisting mainly of pruritus or whealing were prevalent in 41 (85.4%) patients, of whom 16 (33.3%) had moderate or severe symptoms. GI symptoms were present in 33 (68.8%) patients—manifesting as abdominal pain with (29.2%) or without (18.8%) diarrhea and/or peptic symptoms (45.6%). GI symptoms were moderate or severe in 15 (31.3%) patients.

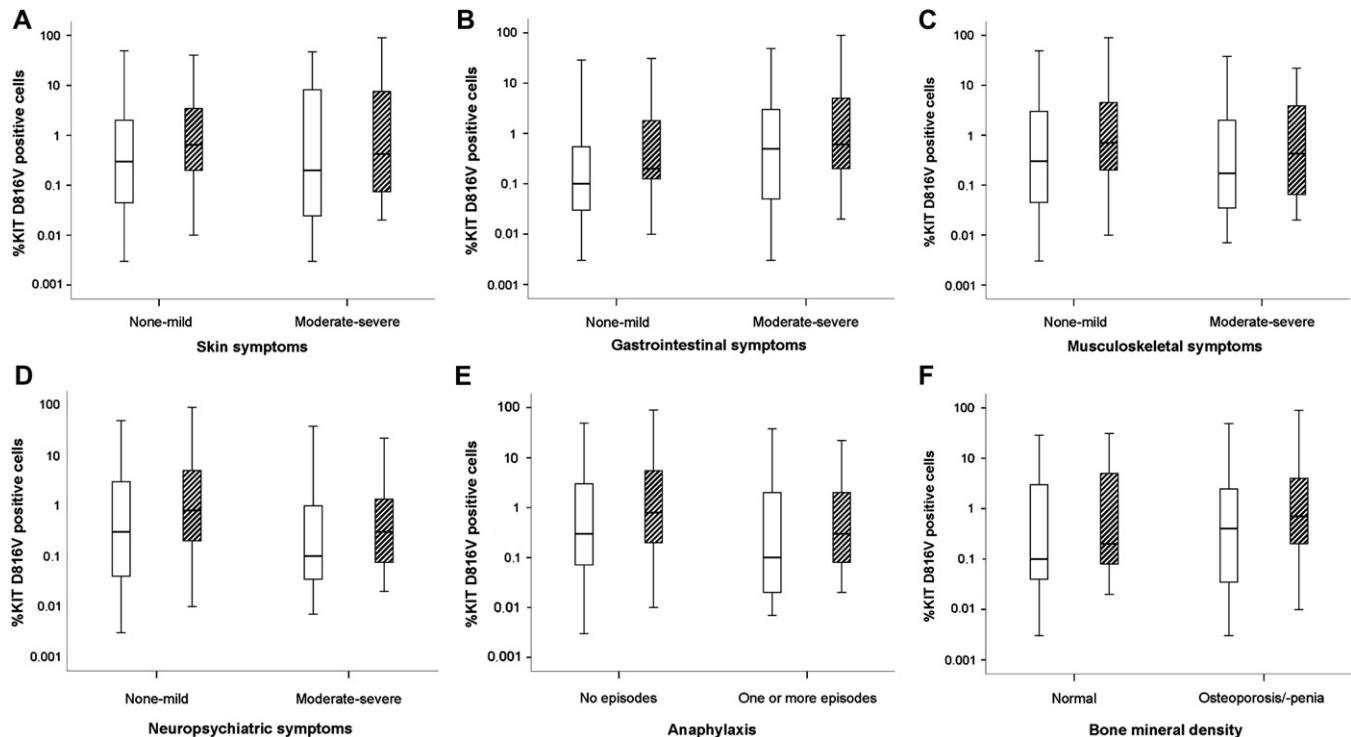


FIG 3. Mutation burden in BM and PB in relation to symptoms, anaphylaxis, and osteoporosis. *White bars* represent PB and *gray bars* BM. **A-D**, Patients divided by severity of symptoms into 2 groups: none to mild or moderate to severe concerning (Fig 3, *A*) skin, (Fig 3, *B*) GI, (Fig 3, *C*) musculoskeletal, and (Fig 3, *D*) neuropsychiatric symptoms. **E**, Patients divided by occurrence of anaphylactic episodes or not. **F**, Patients divided by occurrence of osteoporosis/osteopenia or normal BMD. No significant differences in any of the parameters (Fig 3, *A-F*) can be observed ($P > .05$).

Musculoskeletal pain, primarily originating from the lower back, hips and femora, neck, and shoulders, was seen in 17 (35.4%) patients, of whom 9 (18.8%) had moderate or severe symptoms. Neuropsychiatric symptoms were seen in 15 (31.3%) patients. In 8 (16.7%) cases, these symptoms were moderate or severe and included loss of concentration/memory problems, fatigue, and depressive symptoms. Findings are summarized in Fig 3.

Anaphylaxis

A total of 35.4% of the patients had suffered from 1 or more episodes of anaphylaxis. Elicitors were similar to reports from others (hymenoptera venom, drugs, contrast media, food/alcohol). No significant difference was detected in the fraction of *KIT* D816V mutation-positive cells in patients with and without anaphylaxis. Findings are shown in Fig 3, *E*.

Three patients had experienced 2 or more episodes of anaphylaxis. Separate comparison did not indicate decreased or increased mutation level in these patients compared with the remaining cases.

Osteoporosis

Osteoporosis was found in 14 (29.2%) patients, and osteopenia was found in 18 (37.5%) patients. Three (6.3%) patients had experienced an osteoporotic fracture (2 spine, 1 hip—all females). No significant difference in the mutation level was detected between the group of patients with osteopenia and

osteoporosis and the group of patients with normal BMD ($P = .43$), as shown in Fig 3, *F*. Neither was there a significant difference when analyzing the 3 groups separately ($P = .47$). Among the patients with osteoporosis/osteopenia ($n = 32$), 6 women were postmenopausal opposed to none in the group with normal BMD. No other significant confounders for low BMD (ie, familial disposition, vitamin D insufficiency, smoking habits, steroid usage) were identified ($P > .05$). A total of 8 of 14 (57.1%) patients with osteoporosis and 5 of 18 (27.8%) patients with osteopenia had used antimediator treatment prior to the determination of BMD compared with 6 of 16 (37.5%) patients with normal BMD, without significant differences ($P > .05$).

Median Z score in the entire patient group was -1 SD (-3.7 to -2)—thus, below-average BMD for age-matched healthy individuals. As illustrated in Fig 4, we found no correlation between the fraction of *KIT* D816V mutation-positive cells and Z score (PB: $r = 0.10$, $P = .49$; BM: $r = 0.02$, $P = .79$).

DISCUSSION

In the present study, we demonstrate that the fraction of *KIT* D816V mutation-positive cells in PB or BM does not differ significantly between adults with ISM with different severity of clinical symptoms. We consider this result to be in line with previous data from our laboratory demonstrating that the MCs account for the mutation-positive cell fraction to a varying degree and that the majority of the *KIT* D816V mutated cells in PB and BM do not represent mature MCs.¹⁴ A high and variable proportion of the

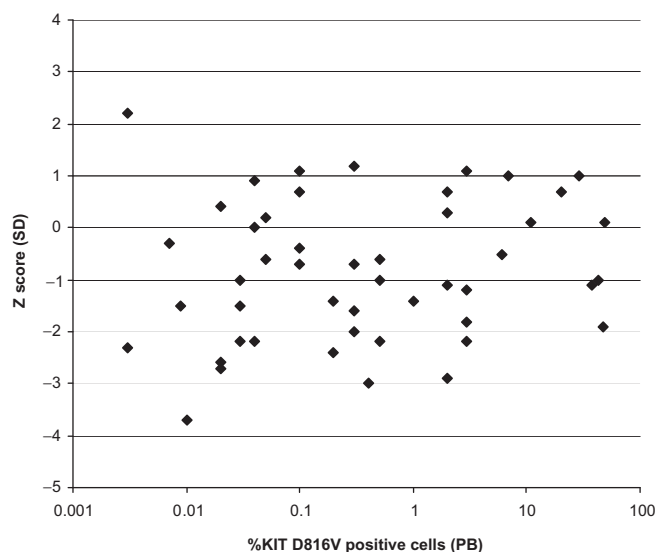


FIG 4. Mutation burden in PB in relation to age-matched BMD (Z score). No significant correlation is seen ($r = 0.10$, $P = .49$). Similar result was found for BM ($r = 0.02$, $P = .79$).

mutation-positive cell fraction is therefore unlikely to contribute to the mediator-related symptoms in ISM and thus probably do not possess the capabilities of the mature MC regarding mediator production and degranulation. Whether this reflects the *KIT* D816V mutation being carried by cells of non-MC lineage or by nonmature MC progenitors is presently not clear, and studies combining highly sensitive *KIT* mutation analysis methods with cell sorting in PB and BM are needed to clarify this.

There is evidence that the *KIT* D816V mutation leads to a hyperactive MC phenotype with a reduced threshold for MC activation.^{1,20,21} The findings in the present study do not contradict this, because the mutation level found is most likely a result of non-MCs and immature MCs carrying the mutation and thus reflecting the clonal expansion of the disease.

The study population in our study overwhelmingly consists of patients with presence of skin lesions. The findings may therefore not apply to patients without skin lesions or to patients with monoclonal mast cell activation syndrome.

We did not find clear evidence for a higher mutation level in patients with longer duration of disease, as should have been expected by the clonal nature of the disease. Time of onset was, however, an estimate in patients in whom firm evidence (skin biopsy/admittance to hospital) was not available, and onset of skin lesions can be inconspicuous. Furthermore, the sample size is relatively small. These uncertainties should caution against drawing firm conclusions; however, a substantial part of the patients studied and even many of those with prolonged duration of disease had a very low mutation level, thus strengthening the general observation of ISM being a stable and slowly progressing disease.

It has been suggested that multilineage involvement of the *KIT* D816V may be a marker for progression of ISM to more severe forms.⁸ In 1998 Worobec and colleagues²² published a study on 65 patients with mastocytosis in whom they detected the *KIT* D816V mutation in PB in 16 (25%) patients, of whom 7 had ISM and 9 had SM with an associated clonal hematologic non-MC disease. The patients in whom the mutation was positive in PB were characterized by older age, longer disease duration,

more severe clinical symptoms including skeletal involvement, hematologic abnormalities, and more severe course of disease. Because the method used for mutation detection in this previous study was less sensitive than the method used in the present study, these patients are likely to have had the highest levels of mutation-positive cells in the study population. Apart from indications of a higher age and disease duration among the subgroup of patients with higher mutation level also found in our study, the previous results represent an apparent contradiction to the present results. However, the conclusion in our study is supported by a detailed analysis of the 7 patients with a mutation level above 10% indicating non-MC and potential multilineage involvement where no significant differences in clinical profile from remaining patients could be observed. Also, in the short period of follow-up, none of these patients had signs of disease progression. Further studies including a larger number of patients with a longer observation period are needed to elucidate whether patients with high mutation levels in fact represent a distinct subpopulation of patients with ISM, and whether the level of mutation may serve as a predictor of the course of disease.

Our study demonstrates that the high level of variation in the fraction of *KIT* D816V mutation-positive cells in PB and BM in patients with ISM does not explain the clinical heterogeneity of the disease. Patients with low mutation levels are just as prone to anaphylaxis, mediator-related symptoms, and osteoporosis as patients with higher levels. This finding may, to some extent, support the clinical practice that cytoreductive treatment is not warranted in most cases of ISM and should mainly be preserved for aggressive forms of the disease, bearing in mind the benign course of ISM with a normal life expectancy and with a low risk of progression to more aggressive forms of SM, thus favoring a treatment strategy focused on relief of symptoms and improving quality of life.

Generally recommended antimediator treatments in ISM include H1 antihistamines and H2 antihistamines, proton pump inhibitors, cromolyn sodium, leukotriene antagonists, short-term glucocorticoids, aspirin/nonsteroidal anti-inflammatory drugs (if tolerated), and avoidance of trigger factors for MC degranulation.⁶ In addition, it is recommended that all patients with SM carry an emergency kit containing epinephrine for self-medication in case of anaphylaxis,⁹ which the findings in the present study support, because one third of included patients had suffered from 1 or more anaphylactic episodes. Treatments used at our center are in line with these general recommendations and most frequently include nonsedating H1 antihistamines (often up-dosed), H2 antihistamines, montelukast, and cromolyn sodium, often combined as add-on therapies—using an individual, tailored approach in each patient depending on the type and severity of symptoms.

The frequency of osteoporosis (29.2%) and osteopenia (37.5%) and osteoporotic fractures (6.3%) in the study population is similar to descriptions from other groups^{11,12} but somewhat higher than that in other published series.⁸ The pathologic mechanism responsible for the development of osteoporosis in SM is not fully understood and is probably multifactorial. In the actual study, we found no evidence that the *KIT* D816V mutation level correlated with BMD. It is possible that MC activation and release of mediators rather than MC burden is of pathogenic importance.²³ Whether reported differences in the frequency of osteoporosis in different series of patients with SM reflect differences in the use of antimediator therapy is not clear. In our study, we did not find differences in the use of antimediator therapy between patients with low BMD and patients with normal

BMD. Further studies are needed to clarify the role of optimal antimediator therapy in prophylaxis and treatment for osteoporosis related to mastocytosis.

In conclusion, here we present the first experimental data from a series of adults with ISM demonstrating that the fraction of *KIT* D816V mutation-positive cells in PB and BM does not correlate with clinical manifestations of the disease. Further studies on a larger number of patients and including the rare aggressive forms of the disease are needed to elucidate the prognostic value and role of mutation level in the management of SM.

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Clinical implications: Level of *KIT* D816V mutation-positive cells is a new variable in SM. In this first study, we found no correlation between mutation level and symptoms, anaphylaxis, or osteoporosis in ISM.

REFERENCES

1. Metcalfe DD. Mast cells and mastocytosis. *Blood* 2008;112:946-56.
2. 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;92:10560-4.
3. 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;12:312-4.
4. Akin C. Clonality and molecular pathogenesis of mastocytosis. *Acta Haematol* 2005;114:61-9.
5. Horny HP, Metcalfe DD, Bennett JM. Mastocytosis. In: Swerdlow SH, Campo E, Harris NL, editors. *WHO classification of tumours of hematopoietic and lymphoid tissues*. 4th ed. Lyon, France: International Agency for Research and Cancer (IARC); 2008. p. 54-63.
6. 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;37:435-53.
7. Pardanani A, Tefferi A. Systemic mastocytosis in adults: a review on prognosis and treatment based on 342 Mayo Clinic patients and current literature. *Curr Opin Hematol* 2010;17:125-32.
8. Escribano L, Alvarez-Twose I, Sanchez-Munoz L, Garcia-Montero A, Nunez R, Almeida J, et al. Prognosis in adult indolent systemic mastocytosis: a long-term study of the Spanish Network on Mastocytosis in a series of 145 patients. *J Allergy Clin Immunol* 2009;124:514-21.
9. 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;63:226-32.
10. Gonzalez de Olano D, de la Hoz Caballer B, Nunez Lopez R, Sanchez Munoz L, Cuevas Agustin M, Dieguez MC, et al. Prevalence of allergy and anaphylactic symptoms in 210 adult and pediatric patients with mastocytosis in Spain: a study of the Spanish network on mastocytosis (REMA). *Clin Exp Allergy* 2007;37:1547-55.
11. Rossini M, Zanotti R, Bonadonna P, Artuso A, Caruso B, Schena D, et al. Bone mineral density, bone turnover markers and fractures in patients with indolent systemic mastocytosis. *Bone* 2011;49:880-5.
12. 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;67:431-8.
13. 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;13:180-8.
14. Kristensen T, Broesby-Olsen S, Vestergaard H, Bindsløv-Jensen C, Moller MB. Mastocytosis Centre Odense University H. Circulating KIT D816V mutation-positive non-mast cells in peripheral blood are characteristic of indolent systemic mastocytosis. *Eur J Haematol* 2012;89:42-6.
15. Zhao W, Bueso-Ramos CE, Verstovsek S, Barkoh BA, Khitamy AA, Jones D. Quantitative profiling of codon 816 KIT mutations can aid in the classification of systemic mast cell disease. *Leukemia* 2007;21:1574-6.
16. Siebenhaar F, Kuhn W, Zuberbier T, Maurer M. Successful treatment of cutaneous mastocytosis and Meniere disease with anti-IgE therapy. *J Allergy Clin Immunol* 2007;120:213-5.
17. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006;117:391-7.
18. Brown SG. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol* 2004;114:371-6.
19. Hanson J. Standardization of femur BMD. *J Bone Miner Res* 1997;12:1316-7.
20. Iwaki S, Spicka J, Tkaczyk C, Jensen BM, Furumoto Y, Charles N, et al. Kit- and Fc epsilonRI-induced differential phosphorylation of the transmembrane adaptor molecule NTAL/LAB/LAT2 allows flexibility in its scaffolding function in mast cells. *Cell Signal* 2008;20:195-205.
21. Akin C, Valent P, Metcalfe DD. Mast cell activation syndrome: Proposed diagnostic criteria. *J Allergy Clin Immunol* 2010;126:1099-104.e4.
22. Worobec AS, Semere T, Nagata H, Metcalfe DD. Clinical correlates of the presence of the Asp816Val c-kit mutation in the peripheral blood mononuclear cells of patients with mastocytosis. *Cancer* 1998;83:2120-9.
23. Kushnir-Sukhov NM, Brittain E, Reynolds JC, Akin C, Metcalfe DD. Elevated tryptase levels are associated with greater bone density in a cohort of patients with mastocytosis. *Int Arch Allergy Immunol* 2006;139:265-70.