Received Date : 05-Oct-2015

Revised Date : 11-Jan-2016

Accepted Date : 18-Feb-2016

Article type : Original Paper

Impact of Centralized Evaluation of Bone Marrow Histology in Systemic Mastocytosis

Mohamad Jawhar[1], Juliana Schwaab[1], Hans-Peter Horny[2], Karl Sotlar[2], Nicole Naumann[1], Alice Fabarius[1], Peter Valent[3], Nicholas C.P. Cross[4,5], Wolf-Karsten Hofmann[1], Georgia Metzgeroth[1], Andreas Reiter[1]

[1] Department of Hematology and Oncology, University Medical Centre Mannheim, Germany
[2] Institute of Pathology, Ludwig-Maximilians-University, Munich, Germany
[3] Department of Internal Medicine I, Division of Hematology and Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, Vienna, Austria
[4] Wessex Regional Genetics Laboratory, Salisbury, U.K.
[5] Faculty of Medicine, University of Southampton, U.K.

Correspondence address:
Prof. Andreas Reiter, M.D.
Department of Hematology and Oncology
University Medical Centre Mannheim
Theodor-Kutzer-Ufer 1-3
68167 Mannheim
Germany
Tel.: +49-621-383-4158
Fax: +49-621-383-4201

E-mail: andreas.reiter@medma.uni-heidelberg.de

Keyword: bone marrow histology, misdiagnosis, ECNM, *KIT* D816V

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/eci.12607

Abstract

BACKGROUND:

Bone marrow (BM) histology/immunohistochemistry, *KIT* D816V mutation analysis and serum tryptase measurements are mandatory tools for diagnosis of systemic mastocytosis (SM).

MATERIALS AND METHODS:

Within the 'German Registry of Disorders on Eosinophils and Mast Cells', we identified 65 SM patients who had two consecutive BM biopsies. The first biopsy was evaluated by a local pathologist (LP), the second biopsy by a reference pathologist (RP) of the ´European Competence Network on Mastocytosis (ECNM)´.

RESULTS:

Final diagnoses by RP were SM (n = 27), SM or aggressive SM (ASM) with associated non-mast cell lineage hematologic disease [(A)SM-AHNMD, n = 34)] or mast cell leukemia ± AHNMD (n = 4). In 15/65 patients (23%), initial diagnoses by LP were incorrect (by overlooking SM), e.g. primary myelofibrosis (n = 3), myelodysplastic/myeloproliferative neoplasm unclassified (n = 3), B-cell lymphoma (n = 2). Fourteen of 15 patients (93%) with incorrect diagnosis had an advanced SM, mostly (A)SM-AHNMD. In the 50 concordantly diagnosed patients, immunohistochemical markers for quantitative assessment of mast cell infiltration, e.g. CD117 (KIT) or CD25, were applied by LP in only 34/50 patients (68%), and mutational analysis for *KIT* D816V was performed or recommended in only 13/50 patients (26%). Finally, the subclassification of SM was discordant because LP did not diagnose AHNMD in 9/50 (18%) patients.

CONCLUSIONS:

In summary, adequate diagnosis and subclassification of SM requires an in-depth evaluation of the BM by experienced hematopathologists (preferably in a reference center) in combination with molecular genetics, serum tryptase and clinical parameters.

Introduction

Systemic mastocytosis (SM) is a rare hematological disorder characterized by an increase and focal accumulation of tissue mast cells (MC) in various organ-systems, predominantly skin, bone marrow (BM) and visceral organs. The type and degree of organ infiltration as well as subsequent organ damage represent the basis for the classification of SM into indolent SM

(ISM), smoldering SM (SSM), SM with an associated clonal hematologic non-MC-lineage disease (SM-AHNMD), aggressive SM (ASM), and mast cell leukemia (MCL) [1-3]. Advanced SM is associated with a poor prognosis, with a median overall survival (OS) of approximately 0.5, 2, and 3.5 years for patients with MCL, SM-AHNMD and ASM, respectively [3]. An acquired mutation in the receptor tyrosine kinase KIT, usually *KIT* D816V, is detectable in over 80-90% of all SM patients [4, 5]. AHNMD usually presents as a myeloid neoplasm such as chronic myelomonocytic leukemia (CMML), myelodysplastic/myeloproliferative neoplasm unclassified (MDS/MPNu) or chronic eosinophilic leukemia (CEL) and frequently reflects the multilineage involvement of *KIT* D816V. The presence of additional mutations, most frequently *TET2*, *SRSF2*, *ASXL1* or *RUNX1*, has recently been reported in a vast majority of patients with advanced SM [6-8]. These mutations usually precede *KIT* D816V and are associated with a more dismal prognosis [7, 9-11].

A thorough histological and immunohistochemical examination of the BM is recommended for the diagnosis and classification of SM. According to the World Health Organization (WHO) classification, diagnosis requires one major (multifocal compact MC infiltration) and one of four minor or three minor criteria, which include 1) atypical MC morphology (> 25% spindle shaped), 2) aberrant MC immunophenotype (expression of CD25/CD2), 3) activating mutations at codon 816 of KIT, predominantly *KIT* D816V, or 4) persistent elevation of baseline serum tryptase levels (> 20 ng/ml) [12].

MC-related immunohistochemical markers, including tryptase and CD117 (KIT), represent powerful tools for the identification and quantification of MCs in the BM or other tissues, which may be more difficult when applying (only) conventional stains, like hematoxylin and

eosin (H&E) or Giemsa, particularly in the presence of an AHNMD (Figure 1) [13]. Moreover, immunostaining enables detection of small but diagnostic compact MC infiltrates in some cases of SM. CD25 is a reliable marker for the differentiation between normal/reactive and neoplastic MCs because aberrant expression of this antigen is highly specific for SM and is seen in almost all patients and subtypes of SM [14]. The diagnosis of AHNMD is established by using WHO criteria, and is confirmed by evaluating peripheral blood (PB) and BM using additional immunological markers such as CD14 (monocytes), CD34 (progenitor/blast cells), CD61 (megakaryocytes), E-cadherin (erythroblasts) and 2D7 (basophils and immature eosinophils) [15]. In this study, we sought to retrospectively evaluate the concordance of diagnosis and classification of SM by comparing morphological, immunohistochemical and molecular analyses in two consecutively performed BM trephine biopsies in 65 patients with *KIT* D816V+ SM.

Materials and methods

Patients

In this retrospective analysis (between 2006-2014), we evaluated 65 SM patients (male 65%, median age 64 years, range 40-85) who had two consecutive BM biopsies. The initial biopsy was evaluated by a local pathologist (LP, mostly academic hematopathologists), while the second biopsy was evaluated by a specialized (reference) pathologist (RP) (H.-P.H. or K.S.) in an ´European Competence Network on Mastocytosis (ECNM)' reference center for hematopathology. The median time between the two biopsies was 6 months (range 1-48). Second biopsies were performed in cases with an insufficient or inaccurate initial report by LP or a clear discrepancy between clinical symptoms, laboratory abnormalities or molecular characteristics and morphologic diagnosis by LP.

Bone marrow histology/immunohistochemistry by RP

Conventional stains, such as Giemsa, Gömöri's silver impregnation and naphthol AS-D chloroacetate esterase were applied to all trephine specimens. Sections were immunostained using the avidin–biotin complex method with antibodies against various antigens associated with MCs (tryptase, CD25, and CD117), stem cells (CD34), myelomonocytic cells (myeloperoxidase, lysozyme, CD15, and CD68), megakaryocytes (CD61), erythroblasts (E-cadherin) and basophils/immature eosinophils (2D7) (Table 1).

For this analysis, SM and ASM were summarized because they can not be differentiated by morphologic findings in the BM but only by the presence or absence of characteristic clinical features (C-findings) which include cytopenia(s) with an absolute neutrophil count < 1 x 10^9/L, hemoglobin < 10.0 g/dL and/or platelets < 100 x 10^9/L, hepatomegaly with impaired liver function, palpable splenomegaly with signs of hypersplenism, malabsorption with significant hypoalbuminemia and/or significant weight loss > 10% over the last 6 months and osteolyses [1].

***KIT* D816V and serum tryptase level**

For the qualitative and quantitative assessment of *KIT* D816V at the RNA-level (expressed allele burden, EAB), allele-specific quantitative real-time polymerase chain reaction analysis (RQ-PCR) was performed in PB or BM as previously described [4]. The presence of *KIT* D816V mutations in BM trephine biopsies was investigated as previously described [16]. Serum tryptase levels were measured by ImmunoCap Tryptase (Phadia Laboratory Systems, Uppsala, Sweden) and were available in 59/65 (91%) patients.

Statistical analysis

All statistical analyses considered clinical and laboratory parameters obtained at time of diagnosis or first referral to our center that in most instances, coincided with time of BM biopsy and study sample collection. For categorical variables, two patient groups were compared with the exact Fisher test. P-values < 0.05 were considered significant. SPSS version 22.0.0 (IBM Corporation) were used for statistical analysis.

Results

Evaluation by RP

Final diagnoses by RP were SM (n = 27), (A)SM-AHNMD (n = 34), MCL (n = 3) and MCL-AHNMD (n = 1). AHNMD included CMML (n = 11), MDS/MPNu (n = 11), CEL (n = 5), myelodysplastic syndrome (MDS, n = 4), primary myelofibrosis (PMF, n = 1), acute myeloid leukemia (AML, n = 1), polycythemia vera (PV, n = 1) and chronic lymphocytic leukemia (CLL, n = 1). All patients with (A)SM/MCL-AHNMD fulfilled the major diagnostic criterion of multifocal dense infiltrates of MCs. Quantitative assessment of the extent of MC infiltration (tryptase, CD117, CD25) was reported in all patients (median 25%, range 5-90). Concomitant reticulin fibrosis and eosinophilia was observed in 41/65 (64%) and 48/65 (74%) patients, respectively. *KIT* D816V mutational analysis was performed and positive in all 65 patients (Table 2). The serum tryptase level (normal value < 11.4 ng/ml) was elevated in 59/59 patients (median 132 ng/ml, range 12-1690).

Evaluation by LP

LP diagnosed SM in 50/65 (77%) patients: SM (n = 33), SM-AHNMD (n = 14) and MCL (n = 3). Diagnosis of AHNMD included CMML (n = 3), MDS (n = 3), PMF (n = 3), MDS/MPNu (n = 2), CEL (n = 1), PV (n = 1) and CLL (n = 1). MC infiltration was

quantified in 34/50 (68%, median 20%, range 5-90) patients. Immunohistochemistry was performed in 48/65 (74%) cases. CD117 (KIT) and CD25 was applied in 43/50 (86%) and 24/50 (48%) cases, respectively. Fibre staining was performed in 45/65 (69%) cases and commonly associated reticulin fibrosis was observed in 32/45 (71%) cases. Eosinophilia was reported in 26/65 (40%) patients. *KIT* D816V mutational analysis was performed or suggested in 13/50 (26%) correctly diagnosed SM patients (Table 2).

Characteristics of discordantly diagnosed patients

Patients who were initially misdiagnosed (by missing SM) by LP (n = 15) experienced a median delay of 11 months (range 0-61 months) before a second diagnostic biopsy was obtained. In 8/15 patients (53%), the two biopsies were within 12 months. Discordant diagnoses by LP included PMF (n = 3), MDS/MPNu (n = 3), autoimmune thrombocytopenia (ITP, n = 2), indolent B-cell lymphoma (B-NHL, n = 2), CMML (n = 1), hypereosinophilic syndrome (HES, n = 1), anemia of chronic disease (ACD, n = 1) or without pathological findings (n = 2) (Figure 2).

Fourteen of 15 patients (93%) with incorrect diagnosis had an advanced SM [(A)SM ± AHNMD, n = 12, MCL ± AHNMD, n=2). Eleven of the 15 (73%) discordantly diagnosed patients had an AHNMD including MDS/MPNu (n = 6), CMML (n = 3), MDS (n = 1) and CEL (n = 1). In two cases, AHNMD (CMML, n = 1; MDS/MPNu, n = 1) was correctly diagnosed by LP but without diagnosis of SM. The subclassification of SM was discordant because diagnosis of AHNMD was missed in 9/50 (18%) patients. The most frequently missed AHNMD were MDS/MPNu (n = 4) and CMML (n = 3) (Figure 2).

Significant differences ($P < 0.001$) between LP and RP regarding misdiagnosis, missing of AHNMD, quantification of MCs, immunohistochemistry, performance of fibre staining and mutational analysis are summarized in Table 2.

Discussion

In our retrospective analysis of 65 patients who have experienced two consecutive BM biopsies, we unraveled an unexpectedly high proportion of discordant diagnoses between local and reference pathologists of the ECNM, predominantly because of missed SM or missed AHNMD in otherwise correctly diagnosed SM. This data clearly emphasizes the importance of adequate immunohistochemical staining with tryptase, CD117 and CD25 for diagnosis of SM and CD34, CD14 plus other markers as indicated by the combination of PB counts (e.g. monocytosis or eosinophilia), serum tryptase and fluorescence activated cell sorting (FACS) analysis for diagnosis of AHNMD. Furthermore, the quantitative assessment of the extent of MC infiltration, which was performed by LP in only around two-thirds of patients, is an important complementary tool for subclassification and monitoring of response.

The performance or even recommendation for mutational testing for *KIT* D816V in correctly diagnosed SM was also unexpectedly low. Qualitative and quantitative assessment of *KIT* D816V is extremely helpful to establish a correct diagnosis of SM, particularly in cases with only ambiguous BM MC infiltration or puzzling morphological findings. In addition, it is also useful for monitoring of response to treatment through recently established, highly sensitive techniques, which allow the detection of *KIT* D816V at levels down to 0.01% [4, 5, 17]. In SM-AHNMD, the *KIT* D816V mutation is usually also identified in other lineages indicating SM-AHNMD as multilineage involvement of *KIT* D816V. The clinical

consequences of non-diagnosed *KIT* D816V+ SM or SM-AHNMD are dismal considering the inferior prognosis of advanced SM on one side and the recently emerging positive results of targeted treatment towards KIT-dysregulated hematopoiesis with tyrosine kinase inhibitors, e.g. midostaurin (PKC412), or allogeneic stem cell transplantation (ASCT) on the other side [3, 19].

The frequently unspecific symptoms in combination with organ dysfunction (e.g. cytopenia, impaired liver function, malabsorption) may explain the broad range of differential diagnoses, including reactive conditions and neoplasms [15]. The complex clinical picture of SM patients highlights that disease-typical symptoms, alone but even more importantly in combinations, such as anaphylaxis plus osteoporosis, cytopenia plus gastrointestinal symptoms, monocytosis plus eosinophilia or eosinophilia plus retroperitoneal lympadenopathy should more frequently lead to the routine estimation of serum tryptase levels and *KIT* D816V mutation status in PB prior to BM biopsy.

Obviously, one could argue that LP and RP should have analyzed the same biopsy and that at least in some of these patients, SM may just have developed shortly prior to the second biopsy. However, the interval between the two biopsies in three cases with incorrect diagnosis was less than 4 weeks and in all 9 patients with incorrect subclassification through missed AHNMD was only 1 to 8 months. All patients had to live with the incorrect diagnosis by LP, independently from the time interval between evaluation by LP and RP. Arguments that LP may have missed diagnosis due to low MC infiltration are counteracted by the fact that median BM MC infiltration in incorrectly diagnosed patients was 30% and advanced SM was diagnosed in 93% of patients. Several patients have even been treated for the incorrectly

diagnosed disease, e.g. bendamustin for indolent lymphoma, romiplostin for autoimmune thrombocytopenia or immunosuppressive treatment for hypereosinophilic syndrome.

Recently, a study of the Spanish Network on Mastocytosis also compared diagnosis and classification of mastocytosis in non-specialized versus reference centres and showed similar results [20]. BM histology/immunohistochemistry, *KIT* D816V mutation analysis and serum tryptase are mandatory tools for diagnosis of SM. Although an elevated serum tryptase level and the presence of *KIT* D816V could easily be identified in PB in the vast majority of SM patients, particularly in advanced disease, both parameters are usually only measured in daily clinical routine following the diagnosis of SM by BM histology/immunohistochemistry. The central role of BM biopsy for diagnosis of SM should therefore be addressed through adequate use of relevant antibodies for identification and quantification of SM and SM-AHNMD. A more frequent evaluation of serum tryptase and *KIT* D816V in PB would however help to reduce failure or delay the diagnosis of SM. Cases with (suspected) SM, based on clinical and/or morphological findings, should therefore always be (re-)assessed by a reference pathologist or ECNM-registered reference center for SM.

Acknowledgments: This work was supported by the 'Deutsche José Carreras Leukämie-Stiftung e.V.' (grant no. DJCLS H 11/03 and 13/05) and the Austrian Science Fund (FWF) grant SFB F4704-B20.

Authorship: MJ, JS, NN, AF and AR performed the laboratory work for the study. JS, GM, WKH, PV and AR provided patient material and information. KS and HPH reviewed the bone marrow biopsies. MJ, JS, PV, WKH, NCPC, AF and AR wrote the paper.

References

1 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.

2 Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB *et al.* Diagnostic criteria and classification of mastocytosis: a consensus proposal. *Leuk Res* 2001;**25**:603-25.

3 Pardanani A. Systemic mastocytosis in adults: 2015 update on diagnosis, risk stratification, and management. *Am J Hematol* 2015;**90**:250-62.

4 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**:81-8.

5 Kristensen T, Vestergaard H and 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.

6 Soucie E, Hanssens K, Mercher T, Georgin-Lavialle S, Damaj G, Livideanu C *et al.* In aggressive forms of mastocytosis, TET2 loss cooperates with c-KITD816V to transform mast cells. *Blood* 2012;**120**:4846-9.

7 Schwaab J, Schnittger S, Sotlar K, Walz C, Fabarius A, Pfirrmann M *et al.* Comprehensive mutational profiling in advanced systemic mastocytosis. *Blood* 2013;**122**:2460-6.

8 Traina F, Visconte V, Jankowska AM, Makishima H, O'Keefe CL, Elson P *et al.* Single Nucleotide Polymorphism Array Lesions, TET2, DNMT3A, ASXL1 and CBL Mutations Are Present in Systemic Mastocytosis. *PloS One* 2012;**7**:e43090.

9 Jawhar M, Schwaab J, Schnittger S, Sotlar K, Horny HP, Metzgeroth G *et al.* Molecular profiling of myeloid progenitor cells in multi-mutated advanced systemic mastocytosis identifies KIT D816V as a distinct and late event. *Leukemia* 2015;**29**:1115-1122.

10 Hanssens K, Brenet F, Agopian J, Georgin-Lavialle S, Damaj G, Cabaret L *et al.* SRSF2-p95 hotspot mutation is highly associated with advanced forms of mastocytosis and mutations in epigenetic regulator genes. *Hematol J* 2014;**99**:830-5.

11 Damaj G, Joris M, Chandesris O, Hanssens K, Soucie E, Canioni D *et al.* ASXL1 but Not TET2 Mutations Adversely Impact Overall Survival of Patients Suffering Systemic Mastocytosis with Associated Clonal Hematologic Non-Mast-Cell Diseases. *PloS One* 2014;**9**:e85362.

12 Valent P HH, Li CY, Longley JB, Metcalfe DD, Parwaresch RM, Bennett JM. Mastocytosis. In: Elaine S. Jaffe NLH, Harald Stein, James W. Vardiman, editor. *Pathology and genetics: Tumours of haematopoietic and lymphoid tissues: World Health Organization (WHO) Classification of Tumours*. Vol. 1. Lyon, France: IARC Press; 2001pp. 291- 302.

13 Horny HP, Sotlar K and Valent P. Mastocytosis: immunophenotypical features of the transformed mast cells are unique among hematopoietic cells. *Immunol Allergy Clin North Am* 2014;**34**:315-21.

14 Sotlar K, Horny HP, Simonitsch I, Krokowski M, Aichberger KJ, Mayerhofer M *et al.* CD25 indicates the neoplastic phenotype of mast cells: a novel immunohistochemical marker for the diagnosis of systemic mastocytosis (SM) in routinely processed bone marrow biopsy specimens. *Am J Surg Pathol* 2004;**28**:1319-25.

15 Horny HP, Sotlar K and Valent P. Differential diagnoses of systemic mastocytosis in routinely processed bone marrow biopsy specimens: a review. *Pathobiology* 2010;**77**:169-80.

16 Sotlar K, Escribano L, Landt O, Mohrle S, Herrero S, Torrelo A *et al.* One-step detection of c-kit point mutations using peptide nucleic acid-mediated polymerase chain reaction clamping and hybridization probes. *Am J Pathol* 2003;**162**:737-46.
17 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**:1223-32.
18 Ustun C, Reiter A, Scott BL, Nakamura R, Damaj G, Kreil S *et al.* Hematopoietic stem-cell transplantation for advanced systemic mastocytosis. *J Clin Oncol* 2014;**32**:3264-74.
19 Gotlib J HC, George T, Akin C, Sotlar K, et al. : Midostaurin (PKC412) Demonstrates a High Rate of Durable Responses in Patients with Advanced Systemic Mastocytosis: Results from the Fully Accrued Global Phase 2 CPKC412D2201 Trial. *Blood* 2014;**124**:21.
20 Sanchez-Munoz L, Morgado JM, Alvarez-Twose I, Matito A, Garcia-Montero AC, Teodosio C et al. Diagnosis and classification of mastocytosis in non-specialized versus reference centres: a Spanish Network on Mastocytosis (REMA) study on 122 patients. *Br J Haematol* 2015; **172**:56-63.

Table 1: Histology/immunohistochemistry and other diagnostic tools in need to reduce inaccurate, failure or delay of diagnosis in patients with (suspected) systemic mastocytosis.

Diagnostic tools
Histology/Immunohistochemistry
▪ Staining (Giemsa, Gömöri's silver impregnation and naphtol AS-D choloacetase esterase)
▪ Various antigens (CD25, CD117, CD34, CD15, CD68, CD61, E-cadherin, 2D7, tryptase myeloperoxidase, lysozyme)
▪ Optional: *KIT* D816V in bone marrow trephine biopsy
Other
▪ Serum tryptase level in peripheral blood
▪ *KIT* D816V in peripheral blood or bone marrow (qualitative and quantitative)

Table 2: Findings of bone marrow (BM) biopsies by local pathologist (LP) and reference pathologist (RP).

BM histology	LP n = 65 (n, %)	RP n = 65 (n, %)	P-value
Final histological diagnosis			
▪ (A)SM	33	27	
▪ (A)SM-AHNMD	**14 (22%)**	**34 (52%)**	< 0.001
▪ MCL	3	3	
▪ MCL-AHNMD	0	1	
▪ other diagnosis (not SM)	**15 (23%)**	**0 (0%)**	< 0.001
Cellularity			
▪ hypercellular	48	54	
▪ normocellular	5	10	
▪ hypocellular	3	1	
▪ not reported	9	0	
Mast cells			
▪ increased	**50 (77%)**	**65 (100%)**	< 0.001
▪ quantified	**34 (68%)**	**65 (100%)**	< 0.001
▪ normal	0	0	
▪ not reported	15	0	
Eosinophils			
▪ increased	**26 (40%)**	**48 (74%)**	< 0.001
▪ normal	0	0	
▪ not reported	39	17	
Immunohistochemistry			
▪ performed	**48 (74%)**	**65 (100%)**	< 0.001
CD117/Tryptase			
▪ positive	43 (86%)	65 (100%)	
CD25			
▪ positive	**24 (48%)**	**65 (100%)**	< 0.001
▪ not performed	24	0	
Fibre staining			
▪ performed	**45 (69%)**	**65 (100%)**	< 0.001
▪ fibrosis	32 (71%)	41 (64%)	
Molecular markers			
▪ *KIT* D816V			
▪ performed (positive: n, %)	**13 (13, 100%)**	**65 (65, 100%)**	< 0.001

BM, bone marrow; PB, peripheral blood.

Figure legends

Figure 1: Systemic mastocytosis, aggressive subtype, with an associated, hematological non-mast cell (myeloid) neoplasm (SM-AHNMD). Extremely hypercellular bone marrow with subtotal depletion of fat cells and diffuse increase in eosinophilic granulocytes at all stages of maturation (Figs. H&E, Giemsa). Immunohistochemistry discloses large compact infiltrates consisting of round mast cells with coexpression of CD25, CD117 (KIT) and tryptase. Note that Tryptase is not expressed by all mast cells and then often to a lesser intensity than CD117 (Figs. CD117, Tryptase). Expression of CD25 defines an aberrant immunophenotype of the mast cells (Fig. CD25). The antibody 2D7 detects a basophil-related antigen and depicts a cell cluster in the immediate vicinity of a compact mast cell infiltrate (Fig. 2D7). The final diagnosis could read as follows: ASM-HES. Altogether, this is a very challenging diagnosis and only possible after thorough immunohistochemical analysis.

Figure 2: Circos diagram: pairwise linking between diagnosis assigned by local pathologist (LP, left) vs. reference pathologist (RP, right). The width of the ribbon/column correlates to the relative frequency of individual diagnosis. ACD, Anemia of chronic disease; B-NHL, B-cell non-Hodgkin lymphoma; (A)SM, (aggressive) systemic mastocytosis; (A)SM-AHNMD, (A)SM with associated clonal hematologic non-mast cell lineage disease; CMML, chronic myelomonocytic leukemia; MDS/MPNu, myelodysplastic/myeloproliferative neoplasm unclassified; HES, hypereosinophilic syndrome; IMF, idiopathic myelofibrosis; ITP, autoimmune thrombocytopenia; MCL, mast cell leukemia; u.a., without pathological findings.