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Published in:
Journal of Allergy and Clinical Immunology: In Practice

DOI:
[10.1016/j.jaip.2020.05.005](https://doi.org/10.1016/j.jaip.2020.05.005)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Schwaab, J., Hartmann, N. C. D. O., Naumann, N., Jawhar, M., Weiss, C., Metzgeroth, G., Schmid, A., Lubke, J., Reiter, L., Fabarius, A., Cross, N. C. P., Sotlar, K., Valent, P., Kluin-Nelemans, H. C., Hofmann, W-K., Horny, H-P., Panse, J., & Reiter, A. (2020). Importance of Adequate Diagnostic Workup for Correct Diagnosis of Advanced Systemic Mastocytosis. *Journal of Allergy and Clinical Immunology: In Practice*, 8(9), 3121-3127.e1. <https://doi.org/10.1016/j.jaip.2020.05.005>

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Importance of Adequate Diagnostic Workup for Correct Diagnosis of Advanced Systemic Mastocytosis

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What is already known about this topic? Little is known about the epidemiology of advanced systemic mastocytosis (SM) given the heterogenic nature of the disease.

What does this article add to our knowledge? We describe epidemiologic aspects and potential pitfalls in the diagnostic workup of advanced SM in Germany contributing to a better understanding and increased awareness of the disease.

How does this study impact current management guidelines? Potential signs of advanced SM should be taken into consideration more frequently in the diagnostic workup of suspected indolent SM. Testing for serum tryptase levels and KIT D816V should be implemented in the routine workup of mastocytosis.

BACKGROUND: Little is known about the epidemiology of advanced systemic mastocytosis (advSM).

OBJECTIVES: To investigate epidemiologic features and diagnostic pitfalls of advSM in Germany.

METHODS: Therefore, 140 patients from a single German reference center of the European Competence Network on Mastocytosis between 2003 and 2018 were analyzed.

RESULTS: The patients' median age was 68 years (range, 26-86 years), and male versus female ratio was 2:1. An elevated serum tryptase, a KIT D816 mutation, and additional somatic mutations, for example, in *SRSF2*, *ASXL1*, or *RUNX1*, were identified in 95%, 91%, and 74% of patients, respectively. Median overall survival was 3.5 years (range, 0.03-14.3 years; male vs female 2.6 vs 4.2 years; $P = .02$). Two categories of misdiagnoses were identified in 51 of 140 (36%) patients: First, systemic mastocytosis (SM) was overlooked in 28 of 140 (20%) patients primarily diagnosed with various subtypes of myeloid neoplasms. Second, 23 of 140 (16%) patients were diagnosed

with supposed progression from indolent SM to advSM; however, combination of an elevated KIT D816V variant allele frequency in peripheral blood ($n = 22$), monocytosis ($n = 9$), eosinophilia ($n = 6$), and/or mutations in *SRSF2*, *ASXL1*, or *RUNX1* ($n = 10$) suggest that distinct signs of potential advSM were overlooked in virtually all patients. Based on locally diagnosed patients in an area of 2.5 million inhabitants, but obviously without considering more, yet unrecognized cases, the incidence and prevalence of advSM is at least 0.8 and 5.2, respectively, per 1 million inhabitants.

CONCLUSIONS: Adequate analyses of tryptase levels, bone marrow morphology, and genetics in patients with myeloid neoplasms or SM would help to prevent the significant underdiagnosis of advSM. © 2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;8:3121-7)

Key words: Epidemiology; Incidence and prevalence of advSM; Misdiagnosis; Multilineage involvement; Disease awareness

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This work was supported by the "Deutsche José Carreras Leukämie-Stiftung e.V." (grant no. 01 R/2018), Germany.

Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication February 25, 2020; revised April 30, 2020; accepted for publication May 4, 2020.

Available online May 15, 2020.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2020.05.005>

Abbreviations used

advSM- Advanced systemic mastocytosis
BM- Bone marrow
CMML- Chronic myelomonocytic leukemia
EAB- expressed allele burden
ECNM- European Competence Network on Mastocytosis
ISM- Indolent systemic mastocytosis
MARS- Mutation-adjusted risk score
MC- Mast cell
MCL- Mast cell leukemia
MPN- Myeloproliferative neoplasm
MDS/MPN- Myelodysplastic/myeloproliferative neoplasm
OS- Overall survival
PB- Peripheral blood
S/A/R- *SRSF2*, *ASXL1*, or *RUNX1*
SM- systemic mastocytosis
SM-AHN- systemic mastocytosis with associated hematologic neoplasm

INTRODUCTION

Systemic mastocytosis (SM) is a rare myeloid neoplasm characterized by abnormal mast cell (MC) proliferation and accumulation in various organs such as bone marrow (BM), visceral organs, and skin.^{1,2} The current World Health Organization classification recognizes SM as a separate myeloid neoplasm.²⁻⁴ Mutations in the *KIT* gene, usually *KIT* D816V, are a pathogenetic and diagnostic hallmark that is present in more than 90% of patients with SM.^{2,5,6} Depending on type and degree of organ infiltration and subsequent organ damage, SM is classified into “nonadvanced” subtypes, for example, indolent SM (ISM) and smoldering SM, and into advanced SM (advSM), which comprises SM with an associated hematologic neoplasm (SM-AHN), aggressive SM (ASM), and mast cell leukemia (MCL).^{1,7}

Although life expectancy is not significantly impacted in patients with ISM,^{8,9} advSM is associated with a poor prognosis, with a median survival ranging between 6 and 18 months in MCL and between 2 and 4 years in SM-AHN and ASM.^{10,11} The poor prognosis of patients with advSM is closely related to the presence and number of additional somatic mutations, for example, in *SRSF2*, *ASXL1*, or *RUNX1* (*S/A/R* gene panel).¹²⁻¹⁴ Most of the recently reported prognostic scoring systems include a combination of clinical characteristics and additional somatic mutations.¹⁴⁻¹⁶

To date, relatively little is known about the incidence and prevalence of SM and its subtypes. A Danish analysis reported an overall incidence of SM (including more than 80% of patients with ISM) of 0.9 per 100,000 per year.¹⁷ Van Doormaal et al¹⁸ reported a prevalence of ISM of 13 per 100,000 inhabitants in the Netherlands. However, since initial reporting, the incidence and prevalence may have risen because of improved disease awareness and more widely available diagnostic tests, particularly serum tryptase and mutation analysis. The European Competence Network on Mastocytosis (ECNM) is focusing on the improvement of diagnosis and treatment of SM and comprises reference centers and centers of excellence all over Europe.^{19,20} Between 2009 and 2018, 140 patients with advSM were referred to our ECNM center from all over Germany. On the

basis of both our local and the overall patient cohort from Germany and through comparison with data from a second and independent ECNM center, we sought to analyze epidemiologic characteristics of advSM.

METHODS

We retrospectively evaluated 140 patients with advSM who were referred, diagnosed, and treated at our ECNM center for MC disorders between January 2009 and December 2018. Classification of patients was based on established World Health Organization criteria.³ All patients gave written informed consent and were registered in the “German Registry on Disorders of Eosinophils and Mast Cells.” The study design adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Medical Faculty of Mannheim, University of Heidelberg.

Mutation analysis for *KIT* D816V on RNA level and next-generation sequencing analysis for additional mutations was performed as previously described.^{6,12}

Statistical analyses were performed using GraphPad prism 8 software (Graphpad Software, San Diego, Calif) or SAS, release 9.4 (SAS Institute Inc, Cary, NC). For qualitative factors, absolute and relative frequencies are given. Quantitative variables are presented by median values together with corresponding ranges. Survival probabilities were calculated by the Kaplan-Meier Method, and correlation between variables was investigated using the Mann-Whitney *U* test (*t* approximation). In general, a test result with *P* less than .05 has been considered as statistically significant.

RESULTS**Sex/age**

There was a significant male predominance (2:1; males, *n* = 94; females, *m* = 46). Median age at diagnosis of advSM was 68 years (range, 26-86 years; female vs male, median, 67.2 vs 69.5 years; *P* = .024). Median distance from hometown to our referral center was 194 km (range, 2-723 km). Distance was less than 50 km in 29 (21%) patients, 50 to 100 km in 15 (11%), 100 to 200 km in 30 (21%), and more than 200 km in 66 (47%) patients. Therefore, more than half the patients came to our center from beyond the close vicinity (Figure 1; see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Patient characteristics are listed in Table I.

Mutations

A mutation in *KIT* was detectable in 136 of 140 (97%) patients, predominantly *KIT* D816V (132 of 140 [94%]). In 96% of *KIT* D816V-positive patients, the mutation could be detected in BM and peripheral blood (PB); only 5 patients who were tested positive in BM were negative in PB using RNA-based quantitative real-time PCR technique.⁶ The median *KIT* D816V expressed allele burden (EAB, ie, quantification of the *KIT* D816V allele burden on the RNA level) in PB was 29% (range, 0%-77%). Two patients (1%) were positive for *KIT* D816H and *KIT* D816Y, respectively. At time of progression from ISM to advSM, a *KIT* mutation was detectable in PB of 22 of 23 (96%) patients (*KIT* D816V, *n* = 21; *KIT* D816H, *n* = 1). Targeted next-generation sequencing was performed in 130 of 140 patients. At least 1 additional mutation was found in 103 of 130 (79%) patients, with a statistically significant imbalance between male and female (males, 76 of 87 [87%] vs females, 27 of

FIGURE 1. Place of residence of all patients with advSM who were referred to Aachen or Mannheim between 2003 and 2018.

43 [63%]; $P = .0057$) patients. In 57 of 76 (75%) male patients and 17 of 27 (63%) female patients, at least 1 mutation in the *S/A/R* gene panel was identified.

Classification of SM

The 140 patients were subclassified as SM-AHN ($n = 105$ [75%]), ASM ($n = 9$ [6%]), and MCL ($n = 26$ [19%]). Interestingly, 17 of 26 patients with MCL presented with MCL-AHN. AHN overall comprised chronic myelomonocytic leukemia (CMML, 44 of 122 [36%]), myelodysplastic/myeloproliferative neoplasm (MDS/MPN unclassifiable, 31 of 122 [25%]), MDS (13 of 122 [11%]), hypereosinophilic syndrome/chronic eosinophilic leukemia, not otherwise specified (12 of 122 [10%]), various subtypes of MPN (12 of 122 [10%]), acute myeloid leukemia (15 of 122 [12%]), chronic lymphocytic leukemia or lymphoma (3 of 122 [2%]), and multiple myeloma (1 of 122 [1%]).

De novo advSM versus advSM with history of ISM

Most patients were diagnosed with *de novo* advSM ($n = 117$ [84%]) without a history of ISM. In 29 patients (21%), BM analysis at initial presentation revealed various hematologic disorders including CMML ($n = 6$), MDS ($n = 5$), MPN ($n = 5$), MDS/MPN ($n = 4$), immune thrombocytopenia ($n = 4$), lymphoma ($n = 2$), reactive conditions ($n = 2$),

hypereosinophilic syndrome ($n = 1$), and 1 patient with metastatic breast cancer ($n = 1$) but failed to identify an underlying SM. Only a repeated investigation led to correct diagnoses median 24 months (range, 1.3-86 months) after the initial diagnosis.

In a cohort of 23 patients, the diagnosis of advSM was established in median 32 months (range, 1-165 months) after the initial diagnosis of ISM ("advSM with history of ISM"). All those patients presented with features of a multilineage and/or multi-mutated myeloid disease process, with measurable *KIT* D816V EAB in PB (22 of 23 [96%]), somatic mutations (14 of 22 [64%]) in the *S/A/R* gene panel (10 of 22 [46%]) or other genes ($n = 4$; *JAK2*, $n = 3$; *TET2*, $n = 3$; *CBL*, $n = 1$; *EZH2*, $n = 1$), and/or alternative features of myeloid proliferation, for example, monocytosis or eosinophilia (13 of 23 [57%]). In the absence of morphologically proven AHN or C-findings, these features are in clear favor of an ISM with multilineage involvement or an overlooked SM-AHN. Most patients therefore presented with SM-AHN or MCL-AHN (17 of 23 [74%]). Interestingly, C-findings were present in only 8 of 23 (35%) patients in this cohort, which may be because the SM component in SM-AHN was often ISM and some of the cases with MCL had chronic MCL without C-findings.

Incidence and prevalence

In the last 15 years, the median number of newly referred patients was 10 per year (range, 0-19; Figure 1) and the median number of patients being managed at our site was 34 (range, 1-74) per year, with higher numbers following the initiation of the midostaurin trial²¹ in 2009 (median number of new referrals, $n = 13$, range, 5-19; ongoing patients, $n = 57$, range, 21-74; Figure 2, A and B).

Because of the significant heterogeneity of referrals from various regions of Germany (map), we subsequently focused for calculation of incidence and prevalence of advSM in Germany on patients from the closer environment of the University Hospital Mannheim, the so-called Metropolitan Region Rhein-Neckar (Figure E1) with 2.4 million inhabitants. On the basis of a total of 30 newly diagnosed patients throughout the whole time period, we calculated an incidence of 0.4 (before 2008) and 0.8 (from 2009), respectively, and a prevalence of 3.3 (overall, range, 1-18) and 5.2 (from 2009, range, 7-18), respectively, per 1 million inhabitants. Approximated current numbers for incidence and prevalence would be 68 and 431, respectively, for the entire region of Germany with 82.8 million inhabitants.

We sought to validate our data in a second, independent ECNM center (University Hospital Aachen, Germany) covering approximately 555,000 inhabitants. Calculated current numbers

TABLE I. Patients' characteristics

SM subtype	n	Characteristic		
		Age (y), median (range)	Serum tryptase level, median (range)	<i>KIT</i> D816 positive, n
ASM	9	65 (27-78)	211 (80-561)	9
SM-AHN	105	69 (26-87)	132 (5-951)*	104
MCL	9	63 (47-75)	628 (193-1,690)	7†‡
MCL-AHN	17	68 (48-86)	371 (61-1,675)	16†‡

*Normal serum tryptase level in 7 patients (5%).

†*KIT* D816Y.

‡*KIT* D816H.

FIGURE 2. (A) Cumulative frequencies of patients newly diagnosed with advSM in Mannheim and Aachen (2003-2018). (B) Cumulative frequencies of patients with advSM being treated in Mannheim and Aachen (2003-2018). AAR, Aachen City Region; MA, Mannheim; MPRN, Metropolitan Region Rhein-Neckar. *Patients.

(2013-2018) for incidence and prevalence are 0.9 and 7, respectively, per 1 million inhabitants, corresponding to an incidence and prevalence of around 75 and 597 for the entire region of Germany. Figure 1 depicts all analyzed patients from Aachen (blue) and Mannheim (red).

Overall survival in various subgroups

Age was a risk factor for inferior survival. Median overall survival (OS) in ASM, SM-AHN, and MCL ± AHN was “not reached,” 3.3 years, and 2.6 years, respectively. OS in male patients was significantly shorter as compared with female

TABLE II. Clinical and laboratory parameters in patients with advSM with and without history of ISM

Patients parameter	Progression of "ISM" to advSM/misdiagnosis of AdvSM		De novo advSM	
	22/23		104/117	
NGS				
S/A/R	Negative	Positive	Negative	Positive
Patients, n (%)	12 (57)	10 (43)	40 (45)	64 (55)
SM-AHN	7	10	32	62
Median time (y) to diagnosis of advSM (range)	5.2 (0.7-38)	2.3 (0.2-13)	NA	NA
C-findings				
Hemoglobin (g/dL), median (range)	12.5 (9.8-15.1)	11.3 (9.2-14.3)	11.1 (5.8-16)	10.7 (6.5-14.2)
<10 g/dL, n (%)	1 (8)	2 (20)	15 (38)	28 (44)
Platelets ($\times 10^9/L$), median (range)	200 (83-441)	123 (18-308)	170 (12-993)	95 (12-795)
<100 $\times 10^9/L$, n (%)	3 (25)	3 (30)	15 (38)	34 (53)
Albumin level (g/L), median (level)	40 (24-45)	38 (32-46)	39 (22-58)	35 (19-48)
<30 g/L, n (%)	1 (8)	0 (0)	4 (10)	7 (11)
Alkaline phosphatase (U/L), median (range)	99 (55-511)	176 (49-621)	127 (50-640)	259 (54-1206)
>126 U/L, n (%)	4 (33)	6 (60)	18 (45)	51 (80)
B-findings				
Serum tryptase level ($\mu g/L$), median (range)	194 (61-1030)	335 (41-952)	124 (5-1690)	187 (24-1675)
>200 $\mu g/L$, n (%)	5 (42)	7 (70)	14 (35)	29 (45)
Splenomegaly, n (%)	12 (100)	8 (80)	30 (75)	55 (86)
Ascites, n (%)	4 (33)	6 (60)	10 (25)	31 (48)
Additional SM and/or AHN-relevant findings				
<i>KIT</i> D816V AB in PB (%), median (range)	28 (12-77)	45 (0-74)	19 (0-67)	31 (0-74)
Leukocytes ($\times 10^9/L$), median (range)	8.2 (3.6-19.3)	9.8 (4.4-66)	11.4 (2.4-89.8)	8.7 (1.3-123.5)
Eosinophils ($\times 10^9/L$), median (range)	0.3 (0.0-1.3)	0.9 (0.0-13.2)	0.3 (0.0-16.3)	0.4 (0.0-100)
>1 $\times 10^9/L$, n (%)	3 (25)	3 (30)	8 (20)	20 (31)
Monocytes ($\times 10^9/L$), median (range)	0.5 (0.2-2.7)	1.2 (0.4-3.8)	0.6 (0-4.1)	1.1 (0.07-17.9)
>1 $\times 10^9/L$, n (%)	3 (25)	5 (50)	9 (23)	32 (50)
Outcome				
MARS, n (%)				
Low	9 (75)	1 (10)	23 (58)	0 (0)
Intermediate	2 (17)	3 (30)	12 (30)	13 (20)
High	1 (8)	6 (60)	5 (12)	51 (80)
Median OS (y)	NR	5.7	10.5	2.5

AB, Allele burden; NA, not applicable; NGS, next-generation sequencing; NR, not reached.

patients (2.6 vs 4.2 years from diagnosis of advSM, $P = .019$; 3.5 vs 10.5 years from diagnosis of SM, $P = .039$).

Patients were classified according to the recently published mutation-adjusted risk score (MARS) for advSM¹⁴: a validated, 5-parameter (1 point each for age >60, hemoglobin <10 g/dL, platelets <100 $\times 10^9/L$, presence of 1 *S/A/R* mutation, presence of >1 *S/A/R* mutation) World Health Organization-independent prognostic score that defines 3 risk groups (low risk, 0-1 point; intermediate risk, 2 points; high risk, 3-5 points) for patients with advSM. The relative proportion of MARS low-risk (23 of 104 [22%] vs 10 of 22 [45%]) and MARS high-risk patients (56 of 104 [54%] vs 7 of 22 [32%]; $P = .026$) and the median OS (3.1 years vs not reached, $P = .015$, data not shown) were significantly different between patients with *de novo* advSM and patients with progression from "ISM." In the latter cohort, *S/A/R*-positive patients had less frequent hematological C-findings (42% vs 60%; Table II) but OS was still inferior compared with *S/A/R*-negative patients.

Concurrent solid cancer

In 32 of 140 (23%) patients, various preceding solid cancers were diagnosed in the skin (basalioma, $n = 4$; melanoma, $n = 2$; epithelial cancer, $n = 1$), urogenital tract (prostate cancer, $n = 6$; renal cell carcinoma, $n = 4$; urothelial carcinoma, $n = 2$; seminoma, $n = 1$), gastrointestinal tract (gastric cancer, $n = 2$; colorectal cancer, $n = 2$; cholangiocarcinoma, $n = 1$), or breast ($n = 4$). During follow-up, 1 patient each developed an MC sarcoma, a cholangiocarcinoma, and a non-Hodgkin lymphoma. Incidence of solid cancer was not increased as compared with an age-matched population.

DISCUSSION

In adult patients, SM is characterized by variable MC infiltration in the BM accompanied by an almost invariable involvement of visceral organs. A *KIT* D816V mutation, most frequently D816V, is present in more than 90% of all patients with SM, whereas an elevated serum tryptase is measurable in

more than 80% of patients with ISM and in almost all patients with advSM.^{6,22,23} However, despite a broad availability of these diagnostic markers, our data suggest that the diagnosis of advSM is critically delayed in at least 30% to 40% of patients and is likely to be completely overlooked in a significant proportion of patients unless cases are referred to a specialized center.²⁴ ISM and MCL are relatively easy to recognize and to differentiate, on the basis of the extent of low/high BM MC infiltration and low/high serum tryptase, respectively, whereas the diagnosis of SM-AHN remains most problematic and controversial.

SM-AHN is basically characterized by multilineage involvement of the *KIT* D816V mutation through which the mutation can be identified at variable frequencies in all myeloid lineages, most frequently in monocytes and eosinophils, but potentially also in erythropoiesis, megakaryopoiesis, and even lymphopoiesis.^{5,25-28} In addition, the vast majority of patients with SM-AHN are positive for additional somatic mutations, for example, in the *S/A/R* gene panel, otherwise also found in related myeloid neoplasms such as CMML or MDS/MPN, acting as very strong adverse prognostic markers.¹²⁻¹⁴ SM-AHN can therefore present with individually highly variable combinations of low or high BM MC burden, low or high levels of serum tryptase, and low or high levels of multilineage and multimutated involvement, most frequently of monocytes and eosinophils. Without staining of BM biopsy slides for tryptase or CD117 (*KIT*) and in the absence of testing for serum tryptase and/or *KIT* D816V, SM is overlooked in at least 5%, probably up to 10% of patients, and particularly in those with CMML, MDS/MPN, or chronic eosinophilic leukemia, not otherwise specified/MPN with eosinophilia. Finally, skin lesions are often absent in advSM. All these issues and caveats can explain why advSM, especially SM-AHN, is often overlooked or diagnosed with a substantial delay.

In our series, diagnosis of SM was initially missed in 20% of patients who were primarily diagnosed with one of these myeloid neoplasms for a median of 2 years. An interesting observation was that in the cohort of patients with advSM with a history of ISM and suspected progression into advSM, an individually variable presence of monocytosis, eosinophilia, high *KIT* D816V EAB in PB, and/or even mutations in the *S/A/R* gene was identified. Every patient in this cohort had at least 1 of these features, thus clearly suggesting the presence of SM with multilineage involvement or even as yet unrecognized (early) advSM rather than typical ISM. The significant difference in OS between *S/A/R*-positive *de novo* advSM and *S/A/R*-positive secondary advSM is based on the significantly higher frequency of relevant C-findings and consequently higher proportion of high-risk patients according to MARS in *S/A/R*-positive *de novo* patients with advSM.

The incidence and prevalence of advSM is unclear due to the rarity of this disorder.^{17,18} Having the status of an ECNM center, our national registry and the availability of clinical trials have led to referral of more than 500 patients with SM within the last 10 years and formed the basis for our epidemiologic studies on 140 patients with advSM. Because the origin of the 140 patients was very heterogeneous, with significantly fewer patients coming from northern and eastern Germany, and because it could be argued that data are biased because of referral of nonlocal patients to specialized centers, we primarily focused our calculations on incidence and prevalence of advSM

on 2 restricted local areas with 2.4 million (Metropolitan Region Rhein-Neckar) and 0.55 million (Aachen City Region) inhabitants, respectively, directly surrounding the ECNM centers in Mannheim and Aachen. A possible explanation for the lower incidence but higher prevalence in Aachen City Region may be a lower number of high-risk patients in the Aachen cohort, best proven by a lower proportion of *S/A/R*-positive patients as compared with the Mannheim cohort (3 of 17 [18%] vs 71 of 123 [58%]). In addition, less frequent thorough diagnosis and referral of older/frail patients may add to an underestimation of the real incidence of advSM. Nonetheless, the incidence and prevalence have constantly risen in both areas, whereas numbers are significantly lower in regions without such centers.

In addition, this series revealed even more relevant data on disease characteristics and epidemiologic aspects. Apart from AHN, concomitant hematologic or solid malignancies were present in 23% of patients, which is higher than the 10% of patients recently reported.²⁹ Five patients had 2 or even more simultaneous or consecutive malignancies apart from SM and AHN, suggesting potential predisposition for the development of various hematologic and/or solid neoplasms in those 5 patients. However, the incidence of solid cancer in general was not increased in the SM cohort as compared with an age-matched population.

But which factors have contributed to the improved disease awareness, when BM histology and serum tryptase have otherwise already been available for several years? The first major step forward was the broad availability of qualitative and quantitative PCR techniques for the identification of mutations in *KIT*, most importantly *KIT* D816V. Quantitative PCR at DNA (variant allele frequency) or RNA/cDNA (EAB) level, if routinely available, allows rapid diagnosis of SM, decisively supporting adequate subclassification and allowing monitoring of disease response.^{6,30,31} The second major step forward was the observation of responses of advSM on *KIT* inhibitors such as midostaurin, and more recently, avapritinib or ripretinib.³² Third, the prognostic relevance of next-generation sequencing-based identification of additional somatic mutations has shown the strong relationship between SM and various other myeloid neoplasms, with somatic mutations such as *S/A/R* representing the genetic complexity and prognostic relevance while *KIT* mutations influence phenotype and disease burden.^{12,13,33} The annual meetings of the ECNM may also have contributed to increased awareness and education of young colleagues. Finally, the establishment of national (eg, Germany Registry on Eosinophils and Mast Cells) and European (ECNM) registries was of pivotal importance, and has also attracted non-European partners.³⁴

We conclude that (1) disease awareness has constantly risen through the establishment of registries and the availability of new therapeutic options, (2) the incidence and prevalence of advSM is significantly higher than currently appreciated and the detection rate is highest in areas with specialized centers, (3) prognosis is inferior in male versus female patients and in *de novo* versus secondary advSM, and (4) beside estimation of BM MC infiltration and serum tryptase, the qualitative and quantitative assessment of the *KIT* D816V EAB or variant allele frequency and additional somatic mutations in patients with SM(-AHN) and all related myeloid neoplasms are of utmost

importance for reliable diagnosis, subclassification, and prognostication of SM.

Acknowledgments

We thank all physicians who contributed clinical data of their patients to the “German Registry on Disorders of Eosinophils and Mast Cells.”

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FIGURE E1. Map of the Metropolitan Region Rhein-Neckar delineating the origin of 30 patients from that area.