Incidence and prognostic impact of cytogenetic aberrations in patients with systemic mastocytosis

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Short title: Cytogenetic aberrations in systemic mastocytosis
Abstract

The clinical behavior of systemic mastocytosis (SM) is strongly associated with activating mutations in KIT (D816V in >80% of cases), with the severity of the phenotype influenced by additional somatic mutations, e.g. in SRSF2, ASXL1 or RUNX1. Complex molecular profiles are frequently associated with the presence of an associated hematologic neoplasm (AHN) and an unfavorable clinical outcome. However, little is known about the incidence and prognostic impact of cytogenetic aberrations. We analyzed cytogenetic and molecular characteristics of 109 patients (KIT D816V+, n=102, 94 %) with indolent (ISM, n=26) and advanced SM (n=83) with (n=73, 88%) or without AHN. An aberrant karyotype was identified in SM-AHN (16/73, 22%) patients only. In patients with an aberrant karyotype additional somatic mutations were identified in 12/16 (75%) patients. Seven of 10 (70%) patients with a poor-risk karyotype, e.g. monosomy 7 or complex karyotype, and 1/6 (17%) patients with a good-risk karyotype progressed to secondary acute myeloid leukemia (n=7) or mast cell leukemia (n=1) within a median of 40 months (range 2-190, \(P=0.04\)). In advanced SM, the median overall survival (OS) of poor-risk karyotype patients was significantly shorter than in good-risk/normal karyotype patients (4 vs. 39 months; hazard ratio 11.7, 95% CI 5.0-27.3; \(P<0.0001\)). Additionally, the shortened OS in patients with poor-risk karyotype was independent from the mutation status. In summary, a poor-risk karyotype is an independent prognostic variable in advanced SM. Cytogenetic and molecular analyses should be routinely performed in all patients with advanced SM±AHN because these investigations greatly support prognostication and treatment decisions.
INTRODUCTION

According to the World Health Organization (WHO) classification, systemic mastocytosis (SM) is a distinct myeloid neoplasm characterized by clonal proliferation and accumulation of abnormal mast cells (MC) in various tissues like skin, bone marrow (BM) or visceral organs (Swerdlow, et al. 2017; Valent, et al. 2001). While patients with indolent SM (ISM) have a normal life expectancy, patients with advanced SM (SM with an associated hematologic neoplasm [SM-AHN], aggressive SM [ASM] or mast cell leukemia [MCL]) have a significantly shortened survival (Lim, et al. 2009; Pardanani, et al. 2009). Somatic gain-of-function point mutations within the receptor tyrosine kinase gene KIT, usually D816V, are detectable in approximately 80–90% of all SM patients (Schwaab, et al. 2013; Soucie, et al. 2012), whereas in advanced SM additional somatic mutations, e.g. in SRSF2, ASXL1 and/or RUNX1 (S/A/R pos), are coexisting with KIT D816V and increase its oncogenic potential. As a consequence, a complex mutational profile has a significant impact on phenotype, response to treatment and prognosis (Jawhar, et al. 2017; Jawhar, et al. 2016b; Jawhar, et al. 2015; Schwaab, et al. 2013). A new risk classification was recently proposed including clinical and molecular parameters (e.g., splenomegaly, elevated alkaline phosphatase [AP], and S/A/R pos) (Jawhar, et al. 2016a).

In contrast to molecular aberrations, no SM-specific cytogenetic aberrations have been identified and there is scant information about the incidence and impact of cytogenetic aberrations. Aberrations reported to date have mainly been found in advanced SM and included trisomies of chromosomes 8 and 9 and deletions involving the long arm of chromosome 20 (del(20)(q11)) (Bernd, et al. 2004; Swolin, et al. 1987; Swolin, et al. 2000; Travis, et al. 1988; Wang, et al. 2013). Cytogenetic aberrations are included in widely applied prognostic scoring systems for other myeloid disorders, for example, in myelodysplastic syndromes (MDS) or primary myelofibrosis (PMF) (Greenberg, et al. 2012; Hussein, et al. 2010). Therefore, we retrospectively analyzed the incidence of cytogenetic aberrations and their prognostic impact in a large group of SM patients.
PATIENTS AND METHODS

Patient characteristics
Clinical and laboratory characteristics of 109 patients with ISM (n=26), and advanced SM (n=83; ASM, [n=3], MCL, [n=8], SM-AHN, [n=62] and MCL-AHN, [n=10]) are summarized in Tables 1 and 2. The study followed the tenets of the Declaration of Helsinki and was approved by the ethics committee of the Medical Faculty Mannheim at Heidelberg University, Germany. All patients are registered within the ‘German Registry on Disorders of Eosinophils and Mast Cells’ and gave written informed consent.

Somatic mutation analyses
For quantitative assessment of KIT D816V expressed allele burden (EAB) of peripheral blood (PB, n=102) samples, we used allele-specific quantitative real-time PCR (qPCR) analyses as previously described (Erben, et al. 2014). For the identification of additional somatic mutations, next-generation deep amplicon sequencing (454 FLX amplicon chemistry, Roche, Penzberg, Germany) (Schwaab, et al. 2013) or next generation sequencing (NGS) by library preparation based on the TruSeq Custom Amplicon Low Input protocol (Illumina) and sequencing on the NextSeq instrument (Illumina, San Diego, CA) was performed to investigate 18 different genes recurrently mutated in myeloid neoplasms. Gene mutations were annotated compared to the reference sequence based on the Ensembl Transcript ID (Ensembl release 74 - Dec 2013).

Cytogenetic analysis
Cells were prepared from cultured BM aspirate specimens (cultivated for 24 h or 48 h) as previously described and were analyzed by G- or R-banding techniques. Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN 2016, McGowan-Jordan 2016).
Statistical analysis

Overall survival (OS) was defined as time from initial diagnosis (in patients with normal karyotype) or from the first aberrant karyotype (in patients with aberrant karyotype) to date of death or last contact. Differences in the distribution of continuous variables between categories were analyzed by the Mann-Whitney U test (for comparison of 2 groups). For categorical variables, Fisher’s exact test was used. OS probabilities were estimated using the Kaplan-Meier method and compared by the log-rank test for univariate analysis. For the estimation of hazard ratios (HRs) and multivariate analysis, the Cox proportional hazard regression model was used. P values, < .05 (2-sided) were considered significant. SPSS version 21.0.0 (IBM Corporation, Armonk, NY) was used for the statistical analysis.

RESULTS

Evaluation of patient characteristics

Relevant SM-associated disease characteristics of patients with advanced SM are shown in Table 2. Elevated laboratory parameters were detected on the serum tryptase level (median 225 μg/L), leukocytes (median 12.3×10^9/L), monocytes (median 1.1×10^9/L), eosinophils (median 2.3×10^9/L) and alkaline phosphatase (median 179 U/L). The median BM MC infiltration, determined by BM immunohistochemistry, was 32% (range 3-95). The median hemoglobin level was 10.5 g/dL. The median KIT D816V AB in PB was 23% (range 0.9-100). Overall, there were no significant differences in laboratory characteristics of patients with a normal vs. aberrant karyotype except for monocytosis (Table 2).

Distribution of cytogenetic and molecular aberrations

Clinical and molecular characteristics of the 109 SM patients are shown in Table 1 and Figure 1. Overall, 104/109 (95%) patients had a mutation in KIT (D816V, n=102 [94%], D816H, n=2). An aberrant karyotype was present in 16 patients; all these patients had advanced SM (SM-AHN, n=12; MCL-AHN, n=4).
Overall, 62/75 (83%) KIT D816-mutated advanced SM patients had at least one additional somatic mutation. The most frequently affected genes were TET2 (n = 31, 40%), SRSF2 (n = 28, 36%), ASXL1 (n = 18, 23%), RUNX1 (n = 11, 13%) JAK2 (n = 11, 13%), CBL (n = 9, 12%) and K/NRAS (n = 9, 12%). Less frequently affected genes (<10%) were IDH1/2, EZH2, U2AF1, ETNK1, NPM1, SF3B1, SETBP1 and DNMT3A. Two or more additional somatic mutations were detected in 52% of patients.

In patients with an aberrant karyotype, at least one additional mutation was identified in 12/16 (75%) patients.

Karyotype and molecular evolution in patients with advanced SM

During follow-up, 8/16 (50%) of patients showed disease progression (Table 3). These patients progressed to SM-acute myeloid leukemia (AML) (6/8, 75%), MCL-AML (1/8, 12.5%) or MCL (1/8, 12.5%). Median time from initial diagnosis to progression was 40 months (range 2-190). If available, karyotype evolution was observed at the time of progression in 6/6 (100%) patients. At time point of progression all patients had an aberrant karyotype and 2/8 (25%) patients showed a gain of a further mutation (TP53 / patient #11 and NPM1 / patient #16) (Table 3). Interestingly, almost all patients (8/9) with SM-AML evolved from previously diagnosed SM with CMML (n=3), MDS/MPNu (n=3) or MPN-eo (n=2). The median blast count in BM was in median 40% (range 25-90).

Survival data depending on cytogenetic and molecular aberrations in advanced SM patients

Similar to other (related) myeloid neoplasms, e.g. MDS or AML, patients were classified according to their aberrant karyotype into two groups. The good-risk group (n=73) included patients with a normal karyotype (n=67) and those with a favorable karyotype [del(5q), n=3; trisomy 8, n=1; del(1q), n=1; del(12p), n=1], while the poor-risk group (n=10) included patients with a complex karyotype (defined as ≥3 abnormalities, n=7), monosomy 7 (n=1) or del(5q) in AML (n=1). In advanced SM, the median OS of patients with a poor-risk karyotype was significantly shorter than in patients with a good-risk karyotype (4 vs. 39 months; hazard ratio [HR] 11.7, 95% CI 5.0-27.3; P<0.0001) (Figure 2).
As previously described by Jawhar et al. 2016 and 2017, mutations in S/A/R had a negative impact on survival independent of clinical/laboratory characteristics and WHO classification. Of the 77 cases that were tested for gene mutations, 6/10 (60%) with a poor-risk karyotype and 33/67 (49%) patients with a good-risk karyotype were S/A/R\textsuperscript{pos}. In the current multivariate analyses including S/A/R status and karyotype, poor-risk karyotype and S/A/R status were independent factors regarding OS (S/A/R\textsuperscript{pos}: HR 3.4, 95% CI 1.6-7.4; \( P = 0.002 \); poor-risk karyotype: HR 16.8, 95% CI 6.6-42.9, \( P < 0.0001 \)).

Significant differences regarding OS were observed when comparing the following 3 groups: good-risk karyotype + S/A/R\textsuperscript{neg} (n=34) vs. good-risk karyotype + S/A/R\textsuperscript{pos} (n=33) vs. poor-risk karyotype (n=10) (Figure 2).

**DISCUSSION**

There is only little information available on the incidence and impact of cytogenetic aberrations on disease phenotype and progression in SM and its various subtypes. In our series of patients who were primarily referred to our hematology center with a previous diagnosis of SM, but were subsequently unselected, 15% of all patients, 19% with advanced SM and 22% of patients with SM-AHN had an aberrant karyotype. The presence of an aberrant karyotype was strongly associated with advanced SM (100%) and with the presence of an AHN (100%), which is in accordance with other previous smaller studies reporting cytogenetic aberrations in SM-AHN (Wang, et al. 2013; Worobec, et al. 1998) and/or MCL (Bauchinger and Mezger 1990; Travis, et al. 1986). Since additional mutations were already present before the detection of the poor-risk karyotype, we could show that a poor-risk karyotype affects the OS independently of mutational status. We also found that: a) chromosomal aberrations most frequently were deletions [del(5q), del(1q), del(12q)], followed by trisomies [+8], monosomies [-7], and complex karyotypes (Table 3, Figure 1) (Swolin, et al. 2000), b) trisomy 8 was detected both as single abnormality in SM-MDS and within complex karyotypes in SM-MDS/myeloproliferative neoplasias (MPN) (Lishner, et al. 1996; Wang, et al. 2013), and c) a majority of patients (56%) had a karyotype evolution during follow-up which is more likely to be a basis for
disease progression, resistance to various treatment modalities and poor prognosis (Lewis, et al. 1987).

The prognostic impact of cytogenetic aberrations in myeloid malignancies is mirrored by various international accepted standard risk scores for MDS (Revised International Prognostic Scoring System, IPSS-R) (Greenberg, et al. 2012), or MPN, especially PMF (Refined Dynamic International Prognostic Scoring System for PMF, DIPSS-plus) (Gangat, et al. 2011). Within the IPSS-R score it is possible to classify 91% of MDS patients in risk groups according to an estimated prognostic impact of the observed chromosomal abnormality. Patients with del(5q), del(11q), del(12p), del(20q), -Y or normal karyotype are associated with a good risk prognostics, whereas deletion or loss of chromosome 7, alterations of chromosome 3 and a complex karyotype strongly indicate an inferior OS. Interestingly, trisomy 8 is classified as an intermediate risk category. These observations could be confirmed by data based on large patient cohorts published to date (Cordoba, et al. 2012; Haase, et al. 2007; Morel, et al. 1993) and the present study on patients with SM-MDS. Therefore, our results indicate that cytogenetic aberrations in SM-MDS or SM-MPN may primarily reflect oncogenic defects attributable to the MDS- or MPN component of the disease.

Because of resistant and rapidly progressive disease followed by poor OS, particularly in S/A/R-pos and/or poor-risk karyotype advanced SM patients, intensive chemotherapy and allogeneic stem cell transplantation (SCT) is recommended as the only curative treatment option for young and fit patients. Allogeneic SCT should be performed early because responses on midostaurin, cladribine and/or myeloid-type induction chemotherapy may only be short-lived. However, only a minority of patients are eligible for allogeneic SCT because of advanced age, SM-related organ damage (e.g. liver cirrhosis), comorbidity and frequently poor response or progression on various treatment options including intensive chemotherapy. In our poor-risk karyotype cohort no patient had a (sustainable) response to midostaurin±cladribine±intensive chemotherapy without (n=7) and with allogeneic SCT (n=3). In this respect, in elderly patients or in patients with resistance to cladribine/midostaurin/intensive chemotherapy, hypomethylating agents in SM-MDS/AML with
poor-risk karyotype (of the AHN) offer the possibility of long-term disease control without necessarily
achieving complete remission and can represent a reasonable alternative even as bridging therapy
before allogeneic SCT.

Our retrospective analysis shows that cytogenetic analyses are performed in only a minority
of patients with advanced SM (83/201; 41%) within the `German Registry on Disorders of Eosinophils
and Mast Cells`. Given the above-mentioned significant results, we conclude that molecular and
cytogenetic analyses should be routinely performed in all patients with advanced SM because they
may more accurately assess risk of disease progression, poor prognosis and greatly support
treatment decisions.

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CONFLICTS OF INTEREST

CH is part owner of the MLL Munich Leukemia Laboratory. MM is employed by the MLL Munich
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