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Splenomegaly, elevated alkaline phosphatase and mutations in the *SRSF2*/*ASXL1*/*RUNX1* gene panel are strong adverse prognostic markers in patients with systemic mastocytosis

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ABSTRACT

We evaluated the impact of clinical and molecular characteristics on overall survival (OS) in 108 patients with indolent (n=41) and advanced SM (advSM, n=67). Organomegaly was measured by magnetic resonance imaging (MRI)-based volumetry of liver and spleen. In multivariate analysis of all patients, an increased spleen volume ≥450mL (hazard ratio [HR], 5.2; 95% confidence interval [CI], [2.1-13.0]; P=0.003) and an elevated alkaline phosphatase (AP; HR 5.0 [1.1-22.2]; P=0.02) were associated with adverse OS. The 3-year OS was 100%, 77%, and 39%, respectively (P<0.0001), for patients with 0 (low-risk, n=37), 1 (intermediate-risk, n=32) or 2 (high-risk, n=39) parameters. For advSM patients with fully available clinical and molecular data (n=60), univariate analysis identified splenomegaly ≥1200mL, elevated AP and mutations in the *SRSF2*/*ASXL1*/*RUNX1* (S/A/R) gene panel as significant prognostic markers. In multivariate analysis, mutations in S/A/R (HR, 3.2 [1.1-9.6]; P=0.01) and elevated AP (HR 2.6 [1.0-7.1]; P=0.03) remained predictive adverse prognostic markers for OS. The 3-year OS was 76% and 38%, respectively (P=0.0003), for patients with 0-1 (intermediate-risk, n=28) or 2 (high-risk, n=32) parameters. We conclude that splenomegaly, elevated AP and mutations in the S/A/R gene panel are independent of the WHO classification and provide the most relevant prognostic information in SM patients.

INTRODUCTION

Systemic mastocytosis (SM) comprises a group of rare myeloid neoplasms characterized by accumulation of mast cells (MC) in various tissues, predominantly bone marrow (BM), skin and visceral organs. Depending on the involvement of other hematopoietic lineages and disease-related organ damage, SM can be categorized into indolent SM (ISM) and advanced SM (advSM). AdvSM includes patients with SM and associated clonal hematologic non-mast cell lineage disease (SM-AHNMD), aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL).[1-4] In contrast to ISM, advSM has a poor prognosis with median survival between few months in patients with MCL and less than 2-4 years in patients with SM-AHNMD and ASM.[5]

Activating mutations in the receptor tyrosine kinase KIT, usually *KIT* D816V, are pathogenetically relevant somatic point mutations, detected in >80-90% of patients with SM.[6,7] The multi-lineage expansion by *KIT* D816V and the *KIT* D816V allele burden (AB) have an important impact on disease phenotype and prognosis.[6,8-10] Furthermore, the presence and number of molecular aberrations, e.g. in *SRSF2*, *ASXL*, or *RUNX1* (S/A/R gene panel), have a strong adverse impact on disease phenotype and OS in patients with advSM..[9,11]

One of several WHO criteria to classify SM includes enlargement of visceral organs, e.g. spleen, liver, or lymph nodes. In addition to high MC burden in the bone marrow (BM), elevated serum tryptase > 200µg/L and signs of dysplasia or myeloproliferation in the BM, organomegaly is an accepted B-finding which supports the diagnosis of SSM. Signs of organ damage (C-findings), e.g. cytopenia(s), hepatomegaly with impaired liver function, palpable splenomegaly with signs of hypersplenism, malabsorption with significant hypoalbuminemia and/or significant weight loss, support the diagnosis of advSM. However, only little is yet known about the prognostic impact of clinical and hematological characteristics such as B- and C-findings.

We therefore sought to evaluate the impact of hepatomegaly and splenomegaly, as objectively measured by magnetic resonance imaging (MRI)-based volumetry, as well as other known clinical and morphological characteristics, on prognosis in SM.

PATIENTS AND METHODS

Diagnosis and classification of SM

The diagnosis of SM was established according to the WHO classification: indolent SM (ISM, n=41) and advSM (n=67; ASM, n=5; SM-AHNMD, n=46; MCL, n=7; MCL-AHNMD, n=9).[1,5,12] Fifty-five patients had an AHNMD, including chronic myelomonocytic leukemia (CMML, n=19), myelodysplastic/myeloproliferative neoplasm unclassified (MDS/MPN-U, n=19), chronic eosinophilic leukemia (CEL, n=9), acute myeloid leukemia (AML, n=5), and myelofibrosis (MF, n=3). BM biopsies were evaluated by reference pathologists of the European Competence Network on Mastocytosis, ECNM (H.-P.H. and K.S.). Cytoreductive treatment in 61/67 patients with advSM included midostaurin (n=14), cladribine (n=5), cladribine following midostaurin or *vice versa* (n=16), midostaurin (n=6) or cladribine (n=2) followed by intensive chemotherapy with (n=2) or without (n=6) allogeneic stem cell transplantation. Ten patients were treated with hydroxyurea (n=6), nilotinib (n=2) or decitabine (n=2).

The diagnosis of ASM was based on the presence of one or more C-findings (cytopenia with an absolute neutrophil count < 1 x 10^9/L, hemoglobin < 10.0 g/dL, 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).[2,5,13] Diagnosis of MCL was based on the presence of at least 20% mast cells in BM smears with or without C-findings. The diagnosis AHNMD was established by evaluating peripheral blood (PB, e.g. monocytosis and/or eosinophilia) and BM by morphology, histology and immunohistochemistry using WHO criteria and a panel of standard-markers, depending on the suspected AHNMD subtype.

In all patients with significant eosinophilia (> 1 x 10^9/L), the presence of *FIP1L1-PDGFRA* or other rearrangements of *PDGFRA*, *PDGFRB*, or *FGFR1* were excluded by cytogenetics (no rearrangement of 4q12, 5q31-33 or 8p11), RT-PCR (no fusion gene), and/or FISH analysis (no rearrangement). 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, as part of the 'German Registry on Disorders of Eosinophils and Mast Cells'. All patients gave written informed consent.

Gene mutation analyses

Analysis for mutations in *KIT* and quantitative assessment of the *KIT* D816V expressed allele burden (EAB) were performed by allele-specific quantitative real-time polymerase as described.[6] Next-generation deep amplicon sequencing by 454 FLX amplicon chemistry (Roche, Penzberg, Germany) was performed to investigate 18 genes recurrently mutated in myeloid neoplasms as previously described.[9]

Magnetic resonance imaging (MRI)-based volumetry

All examinations were performed on a 1.5T whole-body MRI system (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany). Volume measurements of liver and spleen were performed by a resident (J.C.) using a FDA-approved, commercially available, software (Aycan OsiriX pro v.2.06 64-Bit). Results were verified by a board-certified radiologist (D.H.). For volume measurements gadolinium-based, contrast-enhanced, axial T1-weighted sequences were utilized that covered both organs (T1 vibe fs tra; acquisition matrix 384 x 225; slice thickness 3mm; FoV 296 x 379mm). Regions of interest were drawn surrounding the surface of each organ in all slices. Subsequently, volume calculation was performed automatically by the software by interpolating and summing up the planimetric results to a single volume. The abdominal MRI also allowed identification and measurement of ascites and abdominal lymphadenopathy (LNA).

Statistical analyses

All statistical analyses considered clinical and laboratory parameters obtained at time of MRI. OS

analysis was considered from the date of MRI to date of death or last contact. Pearson correlation analysis was performed for the correlation between two parameters, e.g. there was a positive correlation if both parameters had a tendency to increased levels and a negative correlation if one parameter had the tendency to increase, the other to decrease. Differences in the distribution of continuous variables between categories were analyzed by either Mann-Whitney test (for comparison of two groups) or Kruskal-Wallis test (for comparison of three groups). For categorical variables, Fishers's exact test was used. For univariate (including almost all SM-related parameters) and multivariate analyses, receiver operating characteristic (ROC) curve with a time-dependent survival probability were used to identify optimal cut-off points. OS probabilities were estimated with the Kaplan-Meier method and compared by the log-rank test in univariate analysis. For the estimation of hazard ratios (HRs) and multivariate analysis, the Cox proportional hazard regression model was used (backward selection). *P*-values < 0.05 (two-sided) were considered significant. There was no adjustment for multiple testing as all analyses were explorative. SPSS version 22.0.0 (IBM Corporation, Armonk, NY, USA) and SAS version 9.2 (SAS Institute, Cary, NC, USA) were used for statistical analysis.

RESULTS

Patients

The study group included 108 patients with a median age of 64 years (range 27-82). Relevant SM-associated disease characteristics are presented in Table 1. The median BM MC infiltration, determined by BM immunohistochemistry, was 20% (range 5-95), and the median serum tryptase was 127 µg/L (range 13-951, normal value < 11.4). Ninety-eight percent (106/108) of the patients were *KIT* D816V positive, one patient was *KIT* D816H positive, and one patient was negative for *KIT* mutations. The median *KIT* D816V EAB in PB (available in 101/108 patients) was 22% (range 1-99).

Median blood counts were as follows: leukocytes 8.0 × 10^9/L, hemoglobin 12.3 g/dL (< 10 g/dl in 22% of patients), platelets 181 × 10^9/L (< 100 × 10^9/L in 25% of patients), monocytes 0.5 x 10^9/L (> 1

x 10^9/L in 21% of patients) and eosinophils 0.2 x 10^9/L (> 1 x 10^9/L in 19% of patients). Non-hematologic C-findings included hypoalbuminemia (< 35 g/L) in 26% of patients, and elevated AP (> 150 U/L; normal value 40-150) in 41% of patients. Median observation time from the date of MRI was 26 months (range 1-79); 36/108 patients (33%) died in the observation period. The 3-year OS probability of all patients was 64%, with a median OS of 5.4 years.

Magnetic resonance imaging (MRI) findings

In advSM, MRI was performed within 6 months of primary diagnosis in 82% of patients, and in the remaining patients within 12 months of diagnosis. The median volumes of liver and spleen were 2035 mL (range 1034-4265) and 540 mL (range 92-3193), respectively. ROC-analysis identified a spleen volume of 450 mL as optimal volumetric thresholds for all patients and 1200 mL for patients with advSM only. The optimal cut-off for hepatomegaly was 2400 mL for all patients.

Overall, MRI-documented hepatomegaly was observed in 31/108 patients (29%), and splenomegaly in 67/108 patients (62%). Splenomegaly was mild (≥ 450 mL and <1200 mL) in 43/67 patients (64%) and marked (≥ 1200 mL) in 24/67 patients (36%). Ascites and LNA were detected in 37/108 patients (34%), and 52/108 patients (48%), respectively (Table 1). Pearson's analyses showed a strong positive correlation between spleen and liver volume or liver volume and ascites. A strong negative correlation was observed between spleen volume and platelets/hemoglobin or ascites and albumin. Other correlations are shown in Figure 1.

Correlation between spleen volume and clinical phenotype

In ISM (n=41, 38% of all patients), splenomegaly was observed in 8/41 patients (20%). In contrast, splenomegaly (mild or marked) was present in 59/67 (88%) patients with advSM. The comparison between no splenomegaly and any type of splenomegaly (mild or marked) revealed significant differences in almost all parameters, including age, B-findings, C-findings, BM MC infiltration, serum tryptase, and *KIT* D816V EAB (Table 1). When patients with mild and marked splenomegaly were compared, the median platelet count, elevated AP (> 150 U/L), liver volume, and ascites were

significantly different, indicating a more aggressive phenotype in patients with marked splenomegaly (Table 1).

Impact of spleen volume on overall survival

The spleen volume was significantly associated with adverse OS. Pairwise significantly different OS probabilities were observed for the comparison between no splenomegaly (n=41) vs. any splenomegaly (n=67, median not reached vs. 3.1 years, HR 10.7 [2.6-44.7], $P < 0.0001$), no splenomegaly (n=41) vs. mild splenomegaly (n=43, HR 6.9 [1.6-30.1], $P = 0.003$), no splenomegaly vs. marked splenomegaly (n=24, HR 33.9 [4.5-255.4], $P < 0.0001$), and mild splenomegaly vs. marked splenomegaly (HR 2.6 [1.3-5.3], $P = 0.005$). The 3-year OS was 98%, 68%, and 30% for patients with no splenomegaly, mild splenomegaly, and marked splenomegaly, respectively (Table 1, Figure 2A).

Correlation between liver volume, clinical phenotype and overall survival

Hepatomegaly was observed in 3/41 patients (7%) and 28/67 patients (42%) with ISM and advSM, respectively. The comparison between no hepatomegaly and hepatomegaly revealed significant differences regarding almost all parameters, including C-findings, BM MC infiltration, serum tryptase, and *KIT* D816V EAB. Almost all patients (29/31, 94%) with hepatomegaly had any form of splenomegaly (mild or marked) but not *vice versa*.

Univariate and multivariate analyses of SM-related parameters

The results of univariate analyses for multiple clinical and laboratory variables concerning OS were significant as follows: age ≥ 65 (HR 4.1 [1.9-9.0], $P = 0.0002$), cytopenia (hemoglobin < 10 g/dL and/or platelets < 100 x 10^9/L, HR 4.5 [2.2-9.2], $P < 0.0001$), ascites (HR 3.2 [1.6-6.5], $P = 0.004$), elevated AP (> 150 U/L, HR 8.3 [3.4-19.9], $P < 0.0001$), hypoalbuminemia (HR 2.7 [1.4-5.2], $P = 0.002$), markedly elevated serum tryptase (> 200 µg/L, HR 3.6 [1.7-7.3], $P = 0.0002$), hepatomegaly (> 2400 mL, HR 2.5 [1.3-4.9], $P = 0.007$), any splenomegaly (≥ 450 mL, HR 10.7 [2.6-44.7], $P < 0.0001$), LNA (HR 2.2 [1.1-4.6], P = 0.03), multi-lineage expansion (monocytes > 1 x 10^9/L and/or eosinophils > 1 x 10^9/L, HR 3.5 [1.8-6.7], $P < 0.0001$) and *KIT* D816V EAB in PB > 30% (HR 3.3

[1.7-6.5], *P* = 0.0003). In multivariate analyses including all clinical and hematological variables with significant prognostic value in univariate analyses, only splenomegaly ≥ 450 mL (HR 5.2 [2.1-13.0], *P* = 0.003) and elevated AP (HR 5.0 [1.1-22.2], *P* = 0.02) remained independent poor risk factors for OS (Table 2A, Figure 2A). Regarding the comparison of the disparate WHO subgroups, e.g. ISM vs. advSM, ASM vs. MCL, ASM vs. SM-AHNMD, MCL vs. SM-AHNMD, only ISM vs. advSM (*P* < 0.0001) was significant in univariate analysis, but was not significant in multivariate analyses.

In univariate analyses of 60/67 patients with advSM and available mutation status of the S/A/R gene panel, marked splenomegaly (≥ 1200 mL, HR 3.0 [1.4-6.4], *P* = 0.002), elevated AP (> 150 U/L, HR 3.6 [1.6-9.3], *P* = 0.006) and mutations in the S/A/R gene panel (HR 4.3 [1.5-12.4], *P* = 0.003) were significant with regard to OS. In multivariate analysis, elevated AP (HR 2.6 [1.0-7.1], *P* = 0.03) and mutations in the S/A/R gene panel (HR 3.2 [1.1-9.6], *P* = 0.01) remained independent poor risk factors for OS (Table 2B, Figure 2B).

Risk stratification

The risk stratification was designed based on the two independent parameters identified in multivariate analysis, attributing points according to their rounded HR value (Table 2A): 0 point (low-risk, n = 37) for patients without splenomegaly and normal AP, 1 point (intermediate-risk, n = 32) for patients with any splenomegaly or elevated AP, and 2 points (high-risk, n = 39) (Figure 3A) for patients with any splenomegaly and elevated AP. Thus, 39/67 (58%) patients with advSM were classified as high-risk and 28/67 (42%) were classified as low-/intermediate-risk (Figure 4A). The median number of C-findings was significantly higher in high-risk patients. For the cases with available DNA for NGS (74/108, 69%), there was a significantly higher incidence of mutations in the S/A/R gene panel in intermediate- (12/24, 50%) and high-risk (28/35, 80%) patients as compared to low-risk patients (0/15) (P < 0.0001). The 3-year OS probabilities of patients with low-, intermediate- and high-risk were 100%, 77%, and 39%, respectively (*P* < 0.0001, Table 3A, Figure 3A).

In the subgroup of patients with advSM and available mutation status of the S/A/R gene panel, a

prognostic model was established based on the multivariate analysis (Table 2B): 0-1 point (intermediate-risk, n=28) for patients with normal AP and no mutation in the S/A/R gene panel or with elevated AP or a mutation in the S/A/R gene panel; 2 points (high-risk, n=32) for patients with elevated AP and mutations in the S/A/R gene panel. Thus, 28/60 (47%) patients with advSM were classified as intermediate-risk and 32/60 (53%) patients were classified as high-risk (Figure 4B). Furthermore, there was a significant correlation between massive splenomegaly and high-risk patients because 17/21 (81%) patients with massive splenomegaly were classified as high-risk ($P = 0.003$). The median OS probabilities of patients with high- and intermediate-risk were 24 months and not reached with a 3-year OS of 38% and 76%, respectively ($P = 0.0003$, Table 3B, Figure 3B).

DISCUSSION

On basis of univariate and multivariate analyses, we demonstrate that splenomegaly, elevated AP and mutations in the S/A/R gene panel are strong and independent adverse prognostic parameters for OS in patients with SM. Importantly these factors are independent of the WHO classification, and delineate new risk categories for patients with indolent and advSM.

Splenomegaly is one of the most prominent clinical characteristics in patients with MPN and is included in widely applied prognostic scoring systems, e.g. in chronic myeloid leukemia.[14-16] Splenomegaly is not included in established prognostic scoring systems for MF but data from ruxolitinib-based clinical trials have demonstrated an adverse prognostic impact of the initial spleen volume and the lack of spleen volume reduction on treatment.[17-19] In MF, these associations were only established because the spleen volume was accurately measured by volumetry through computer tomography (CT) or MRI.

Splenomegaly and/or hepatomegaly and the related organ damage are also considered as clinically relevant parameters in patients with SM. Although splenomegaly is not part of the WHO diagnostic criteria, it is an important criterion for the classification of SM through B- and C-findings as well as

identifying disease progression. However, the prognostic value of splenomegaly has not yet been thoroughly evaluated. Marked reduction of spleen volume as assessed by CT/MRI has recently also been reported for patients with advSM on midostaurin (PKC412), rendering it a valid and reliable parameter for the assessment of response to treatment. Accordingly, an IWG-MRT (ELN and ECNM) expert panel included spleen response in MF and SM in their response criteria. The panel recognized the highly subjective nature of the assessment of spleen and liver size by physical examination, and therefore recommended objective confirmation by CT/MRI.[20,21]

In comparison to splenomegaly, hepatomegaly was significantly less frequently observed in patients with advSM, and only rarely in ISM. Of note, hepatomegaly was strongly associated with the presence of splenomegaly but not *vice versa*. Low albumin, increased liver enzymes and/or portal hypertension with splenomegaly and ascites characterize hepatomegaly with dysfunction of the liver. However, little is known about distinct differences between the various laboratory parameters, e.g. albumin, AST, ALT, AP, GGT and bilirubin.[22] In our patient cohort, AST, ALT, and bilirubin were only elevated in a minority of patients while elevated AP and to a lesser extent also elevated GGT were identified in the vast majority of patients with advSM. In subsequent analyses, elevated AP had a significantly stronger impact on OS as compared to elevated GGT. Although the associated increase of AP and GGT already indicated the source of AP being hepatic but not osseous, AP isoenzyme analyses were performed in 25 patients proving the predominant hepatic origin in 24/25 patients. In addition, all 9 patients with histologically confirmed MC infiltration had an elevated AP (data not shown).

We have recently shown the strong adverse impact of mutations in the S/A/R gene panel on OS in patients with advSM.[11] We therefore included mutation profiles in our analyses and delineated intermediate- and high-risk subgroups of advSM patients based on elevated AP and S/A/R mutations. An SM patient at highest-risk (median survival of 24 months) is characterized by presence of an AHNMD, marked splenomegaly, elevated AP and mutations in the S/A/R gene panel. Of note, all these parameters are easily accessible and serial measurements may thus assist in determining

responses to anti-neoplastic therapy.[23] Because patient numbers are limited, we are aware of that parameters that were excluded in the current model may become more significant in larger series.

In conclusion, splenomegaly, elevated AP and mutations in the S/A/R gene panel provide highly relevant prognostic information for all subvariants of SM, independently of other clinical, hematological and laboratory parameters and also the WHO classification. Similar to other subtypes of myeloid neoplasms, it is likely that clinical and molecular markers complement each other and will have major implications on forthcoming prognostic scoring systems on the basis of larger patient cohorts, such as the dataset of the ECNM registry, with the long-term objective to develop an IPSS for patients with SM.

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FIGURE LEGENDS

Figure 1: The heat map displays correlations between two individual parameters. Colors were assigned on basis of the value of the *Pearson's correlation coefficient (r). E.g., a strong positive correlation (the other variable has a tendency to increase, in blue) was observed between spleen volume and liver volume or liver volume and ascites while a strong negative correlation (the other variable has a tendency to decrease, in red) was observed between spleen volume and platelets/hemoglobin or between ascites and albumin. AB, allele burden; AP, alkaline phosphatase; Hb, hemoglobin; LNA, lymphadenopathy; MC, mast cell infiltration; MLE, multi-lineage expansion; PLT, platelets. **All correlations are statistically significant with the exception of the correlation between LNA and tryptase.

Figure 2: (A) Kaplan-Meier estimates of overall survival (OS) of patients depending on the spleen volume and alkaline phosphatase (AP) in 108 patients with systemic mastocytosis (SM): spleen volume < 450 mL (n=41, blue line) vs. spleen volume ≥ 450 mL and < 1200 mL (n=43, yellow line) vs. spleen volume ≥ 1200 mL (n=24, red line); normal AP (≤ 150 U/L, n=64, blue line) and elevated AP (> 150 U/L, n=44, red line). (B) Kaplan-Meier estimates of OS of patients depending on the spleen volume and AP in 60 patients with advanced SM and known mutation profile in the *SRSF2*/*ASXL1*/*RUNX1* (S/A/R) gene panel; spleen volume < 1200 mL (n=39, yellow line) vs. spleen volume ≥ 1200 mL (n=21, red line); normal AP (≤ 150 U/L, n=22, blue line) vs. elevated AP (> 150 U/L, n=38, red line). Significantly different OS probabilities were observed for the pairwise comparison. The P-values refer to log-rank tests and the HR (hazard ratio) is presented together with the 95% confidence interval.

Figure 3: (A) Kaplan-Meier estimates of overall survival (OS) depending on the independent prognostic markers (alkaline phosphatase [AP] and spleen volume) in multivariate analyses of 108 systemic mastocytosis (SM) patients: low-risk (n=37, blue line), intermediate-risk (n=32, yellow line)

and high-risk (n=39, red line). **(B)** Kaplan-Meier estimates of OS depending on the independent prognostic markers (AP and mutations in the *SRSF2*/*ASXL1*/*RUNX1* [S/A/R] gene panel) in multivariate analyses of 60 with advanced SM: intermediate-risk (n=28, yellow line) and high-risk (n=32, red line). Pairwise significantly different OS probabilities were observed for the comparison between each groups. The P-values refer to log-rank tests and the HR (hazard ratio) is presented together with the 95% confidence interval.

Figure 4: (A) Left column: the WHO based classification of 108 patients with indolent systemic mastocytosis (SM) (n=41) and advanced SM (n=67; aggressive SM [ASM], mast cell leukemia [MCL], SM with associated hematologic non-mast cell lineage disorder [SM-AHNMD] and MCL-AHNMD). **Right column:** advanced SM patients (n=67) were stratified into low- (n=5), intermediate- (n=23) and high-risk (n=39) according to overall survival (OS). (B) **Left column:** the WHO based classification of 60/67 patients with advanced SM and known mutation profile in the *SRSF2*/*ASXL1*/*RUNX1* (S/A/R) gene panel. **Right column:** advanced SM patients were stratified into intermediate- (n=28) and high-risk (n=32) regarding OS.

Table 1: Clinical, laboratory and magnet resonance imaging (MRI) derived[#] volumetric analysis and findings of 108 patients with systemic mastocytosis, stratified by spleen volume < 450 mL (no splenomegaly), ≥ 450 mL and < 1200 mL (mild splenomegaly) or ≥ 1200 mL (marked splenomegaly).

Variables	All patients (n = 108)	Spleen volume < 450 mL (n = 41)	Spleen volume ≥ 450 mL and < 1200 mL (n = 43)	Spleen volume ≥ 1200 mL (n = 24)	*P** Spleen volume < 450 mL vs. ≥ 450 mL	*P** Spleen volume ≥ 450 mL and < 1200 mL vs. ≥ 1200 mL
Age, in years, median (range)	64 (27-82)	50 (34-81)	69 (27-82)	71 (46-81)	< 0.0001	n.s.
Male, n (%)	57 (53)	16 (40)	25 (58)	16 (67)	0.01	n.s.
Diagnosis						
ISM, n (%)	41 (37)	33 (81)	7 (16)	1 (4)	< 0.0001	n.s.
ASM, n (%)	5 (5)	3 (7)	2 (5)	0	n.s.	n.s.
MCL, n (%)	7 (6)	0	6 (14)	1 (4)	0.04	n.s.
SM-AHNMD, n (%)	46 (43)	3 (7)	27 (63)	16 (67)	< 0.0001	n.s.
MCL-AHNMD, n (%)	9 (8)	1 (2)	0	8 (33)	n.s.	0.0001
C-findings						
Hemoglobin, g/dL; median (range)	12.3 (6.8-15.6)	13.7 (9.6-15.6)	11.2 (7.5-15.1)	11.1 (6.8-13.7)	< 0.0001	n.s.
< 10 g/dL, n (%)	24 (22)	1 (2)	14 (33)	9 (38)	< 0.0001	n.s.
Platelets, x 10^9/L; median (range)	181 (26-993)	258 (70-632)	123 (38-993)	101 (26-227)	< 0.0001	0.03
< 100 x 10^9/L, n (%)	27 (25)	1 (2)	14 (33)	12 (50)	< 0.0001	n.s.
Albumin level, g/L; median (range)	39 (22-48)	41 (30-48)	37 (24-45)	36 (22-43)	0.001	n.s.
< 35 g/L, n (%)	28 (26)	3 (7)	15 (35)	10 (42)	0.0002	n.s.
Alkaline phosphatase, U/L; median (range)	117 (40-1736)	73 (42-387)	135 (40-926)	250 (99-1736)	< 0.0001	0.01
> 150 U/L, n (%)	44 (41)	5 (12)	19 (44)	20 (83)	< 0.0001	0.004
Ascites, n (%)[#]	37 (34)	1 (2)	18 (42)	18 (75)	< 0.0001	0.01
B-findings						
MC infiltration in BM, %; median (range)	20 (5-95)	10 (5-80)	30 (5-95)	30 (10-95)	< 0.0001	n.s
> 30 %, n (%)	36 (33)	8 (10)	17 (40)	11 (46)	0.006	n.s.
Serum tryptase, µg/L; median (range)	127 (13-1854)	49 (4-545)	185 (13-1854)	309 (40-1300)	< 0.0001	n.s.
> 200 µg/L, n (%)	40 (37)	4 (10)	21 (49)	15 (63)	< 0.0001	n.s.
Spleen volume, mL; median (range)[#]	540 (92-3193)	214 (92-399)	754 (441-1055)	1575 (1210-3193)		
Liver volume, mL; median (range)[#]	2035 (1035-4265)	1647 (1035-2814)	2116 (1167-4265)	2725 (2134-3741)	< 0.0001	< 0.0001
> 2400 mL, n (%)	31 (29)	2 (5)	11 (26)	18 (75)	< 0.0001	0.0002
Lymphadenopathy***[#]	52 (48)	7 (17)	25 (58)	20 (83)	< 0.0001	n.s.
Other relevant findings						
Monocytes, x10^9/L; median (range)	0.5 (0.1-23.9)	0.4 (0.1-1.8)	0.5 (0.1-23.9)	0.8 (0.2-3.8)	0.003	n.s.
> 1 x 10^9/L, n (%)	23 (21)	1 (2)	14 (33)	8 (33)	< 0.0001	n.s.
Eosinophils, x10^9/L; median (range)	0.2 (0.0-100.5)	0.1 (0-1.5)	0.6 (0-100.5)	0.3 (0-12.2)	< 0.0001	n.s.
> 1 x 10^9/L, n (%)	20 (19)	2 (5)	13 (30)	5 (21)	0.001	n.s.
KIT D816V$^+$ EAB in PB, %; median (range)	22 (1-99)	4 (1-33)	26 (5-65)	38 (10-74)	< 0.0001	n.s.
> 30 %, n (%)	33 (31)	1 (2)	18 (42)	14 (58)	<0.0001	n.s.
Outcome						
Deaths, n (%)	36 (33)	2 (5)	15 (35)	19 (79)		
2-year OS, %	79	98	74	54	**0.003	**0.005

*The *P*-values refer to the Mann–Whitney U test, Fisher's exact test or the **log-rank tests comparing patients with spleen volume < 450 mL and ≥ 450 mL or ≥ 450 mL < 1200 mL and ≥ 1200 mL. ***1 lymph node > 10 mm or > 10 lymph nodes < 10 mm. [#]MRI derived volumetric analysis and findings. Abbreviations: AHNMD, associated hematologic non-mast cell lineage disease; ASM, aggressive SM; EAB, expressed allele burden; ISM, indolent SM; MC, mast cells; MCL, mast cell leukemia; MRI, magnet resonance imaging; n.k., not known; n.s., not significant; OS, overall survival; PB, peripheral blood; SM, systemic mastocytosis.

Table 2A: Univariate and multivariate analyses regarding the prognostic impact of clinical and hematological characteristics on overall survival of 108 patients with systemic mastocytosis.

	Univariate		Multivariate		Prognostic Points
	HR [95% CI]	*P**	HR [95% CI]	*P**	
Age ≥ 65 years	4.1 [1.9-9.0]	0.0002			
C-findings					
Cytopenia**	4.5 [2.2-9.2]	< 0.0001			
Albumin < 35 g/L	2.7 [1.4-5.2]	0.002			
Alkaline phosphatase > 150 U/L	8.3 [3.4-19.9]	< 0.0001	5.2 [2.1-13.0]	0.003	1
Ascites	3.2 [1.6-6.5]	0.004			
B-findings					
MC infiltration in BM > 30 %		n.s.			
Serum tryptase > 200 µg/L	3.6 [1.7-7.3]	0.0002			
Hepatomegaly > 2400 mL	2.5 [1.3-4.9]	0.007			
Splenomegaly ≥ 450 mL	10.7 [2.6-44.7]	< 0.0001	5.0 [1.1-22.2]	0.02	1
Lymphadenopathy	2.2 [1.1-4.6]	0.03			
Other relevant parameters					
Multi-lineage expansion***	3.5 [1.8-6.7]	< 0.0001			
KIT D816V^{+} EAB in PB > 30%	3.3 [1.7-6.5]	0.0003			

Table 2B: Univariate and multivariate analyses regarding the prognostic impact of clinical, hematological and molecular characteristics on overall survival of 60 patients with advanced systemic mastocytosis.

	Univariate		Multivariate		Prognostic Points
	HR [95% CI]	*P**	HR [95% CI]	*P**	
Age ≥ 65 years		n.s.			
C-findings					
Cytopenia**		n.s.			
Albumin < 35 g/L		n.s.			
Alkaline phosphatase > 150 U/L	3.6 [1.6-9.3]	0.006	2.6 [1.0-7.1]	0.03	1
Ascites		n.s.			
B-findings					
MC infiltration in BM > 30 %		n.s.			
Serum tryptase > 200 µg/L		n.s.			
Hepatomegaly > 2400 mL		n.s.			
Splenomegaly ≥ 1200 mL	3.0 [1.4-6.4]	0.002			
Lymphadenopathy		n.s.			
Other relevant parameters					
Multi-lineage expansion***		n.s.			
KIT D816V^{+} EAB in PB > 30%		n.s.			
Mutation in S/A/R	4.3 [1.5-12.4]	0.003	3.2 [1.1-9.6]	0.01	1

The hazard ratio (HR) is presented together with the 95% confidence interval (CI). *The *P*-values refer to the log-rank tests. **Hemoglobin < 10 g/dL and/or platelets < 100 x 10^9/L. ***Monocytes > 1 x 10^9/L and/or eosinophils > 1 x 10^9/L. Abbreviations: BM, bone marrow; EAB, expressed allele burden; MC, mast cell; n.s., non-significant; PB, peripheral blood, S/A/R, *SRSF2*/*ASXL1*/*RUNX1* gene panel.

Table 3A: Clinical characteristics and outcome of 108 patients with several subtypes of systemic mastocytosis, stratified by low, intermediate and high risk based on presence or absence of splenomegaly and/or elevated alkaline phosphatase.

Variables	All patients (n = 108)	Low risk (n = 37)	Inter-mediate risk (n = 32)	High risk (n = 39)	*P** Low risk vs. intermediate and high risk	*P** Intermediate risk vs. high risk
Diagnosis						
ISM, n (%)	41 (37)	32 (87)	9 (28)	0	< 0.0001	0.0003
ASM, n (%)	5 (5)	2 (5)	2 (6)	1 (3)	n.s.	n.s.
MCL, n (%)	7 (6)	0	6 (19)	1 (3)	0.04	0.04
SM-AHNMD, n (%)	46 (43)	2 (5)	15 (47)	29 (74)	< 0.0001	0.001
MCL-AHNMD, n (%)	9 (8)	1 (3)	0	8 (23)	n.s.	0.007
Number of C-findings						
0, n (%)	44 (41)	35 (95)	9 (28)	0	< 0.0001	0.0003
1, n (%)	22 (20)	2 (5)	16 (50)	4 (10)		
2, n (%)	12 (11)	0	4 (13)	8 (21)		
≥ 3, n (%)	30 (28)	0	3 (9)	27 (69)	< 0.0001	< 0.0001
Molecular profile**						
Mutation in S/A/R, n (%)	40 (37)	0	12 (50)	28 (80)	< 0.0001	0.01
Mutation (but not in S/A/R), n (%)	11 (10)	2 (5)	6 (25)	3 (9)		
No mutated gene, n (%)	23 (21)	13 (35)	6 (25)	4 (11)	n.s.	n.s.
Outcome						
Death, n (%)	36 (33)	0	8 (25)	28 (72)		
2-year OS (%)	77	100	81	56	**0.009	**0.001

Table 3B: Spleen volume characteristics and outcome of 60 patients with advanced systemic mastocytosis, stratified by intermediate and high risk based on presence or absence of elevated alkaline phosphatase and/or mutation in *SRSF2*/*ASXL1*/*RUNX1* gene panel.

Variables	All patients (n = 60)	Intermediate risk (n = 28)	High risk (n = 32)	*P** Intermediate risk vs. high risk
Diagnosis				
ASM, n (%)	5 (8)	5 (18)	0	0.02
MCL, n (%)	6 (10)	6 (21)	0	0.008
SM-AHNMD, n (%)	40 (67)	15 (54)	25 (78)	n.s.
MCL-AHNMD, n (%)	9 (15)	2 (7)	7 (22)	n.s.
Spleen volume				
No splenomegaly (< 450 mL), n (%)	7 (12)	5 (18)	2 (6)	n.s.
Splenomegaly (≥ 450 mL), n (%)	32 (53)	19 (68)	13 (41)	0.04
Splenomegaly (≥ 1200 mL), n (%)	21 (35)	4 (14)	17 (53)	0.003
Outcome				
Death, n (%)	31 (52)	7 (25)	24 (75)	
2-year OS (%)	65	79	53	**0.0003

*The *P*-values refer to Fisher's exact test or the **log-rank test comparing patients with low risk vs. intermediate risk/high risk and intermediate risk vs. high risk. **data available in 69% (74/108) of patients. Abbreviations: AHNMD, associated hematologic non-mast cell lineage disease; ASM, aggressive SM; ISM; indolent SM; MCL, mast cell leukemia; OS, overall survival; SM, systemic mastocytosis; S/A/R, *SRSF2*/*ASXL1*/*RUNX1* gene panel.