Presence of neoplastic mast cells in ascites in advanced systemic mastocytosis

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Clinical Implications

In patients with advanced systemic mastocytosis and ascites, neoplastic mast cells expressing CD25 and CD2 can be detected in ascites. These mast cells in ascites might serve as a diagnostic and treatment response marker in advanced systemic mastocytosis.

Systemic mastocytosis (SM) is characterized by clonal expansion of mast cells (MCs) in different organs, usually in bone marrow (BM) and variably also in other tissues like skin and gastrointestinal tract.[1-3] Clinical manifestations are heterogeneous and can include MC mediator symptoms and a dysfunction of various organ systems, such as cytopenia, hepatosplenomegaly, ascites, and weight loss. Most patients with SM carry the activating mutation *KIT* D816V. Typically, patients with SM are also characterized by elevated serum levels of tryptase, an enzyme produced by MCs, and aberrant expression of CD25 and/or CD2 on MCs in BM and possibly other tissues.[1-3] Advanced SM (advSM) is categorized into 3 subtypes: aggressive SM (ASM), SM with associated hematological neoplasm (SM-AHN), and MC leukemia. Here, we report 2 patients with advSM with ascites, in whom we observed neoplastic MCs expressing CD25 and CD2 in the ascites.

After institutional review board approval, routine diagnostic workup of BM biopsies was performed to assess World Health Organization criteria of SM by histochemistry, immunohistochemistry using antibodies against CD117, tryptase, CD25, and CD2, and *KIT* mutational analysis using digital droplet polymerase chain reaction.[1,2] Serum tryptase levels were measured using a fluoroimmunoenzymatic assay (ImmunoCAP Tryptase; Thermo Fisher Scientific, Uppsala, Sweden). In addition, ascites was collected and analyzed by flow cytometry using antibodies against CD45, CD117, CD25, and CD2, by *KIT* mutational analysis, and by measurement of tryptase levels. The study was approved by the local ethics committee (ethics committee of Northwestern and Central Switzerland, Basel, Switzerland) and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained in each case before BM and ascites samples were analyzed.

The first patient was a 72-year-old man presenting with fatigue, weight loss, hepatosplenomegaly, and ascites developing for 5 months. Diagnostic workup of blood and BM as well as recording of C-findings of advSM revealed the diagnosis of ASM (Table I). Additional next-generation sequencing (NGS) of BM could detect mutations in *SRSF2*, *TET2*, and *RUNX1*. Rectal bleeding required endoscopy and abdominal contrast-enhanced computed tomography, which then also detected a locally advanced rectal adenocarcinoma. We ruled out significant heart, liver, and kidney diseases as causes of the ascites. Analysis of ascites revealed a transudate without metastatic cells, but with, interestingly, CD117-positive MCs expressing CD25 and CD2 (Table I, Figure 1). Some of these MCs also showed a spindle-shaped phenotype (data not shown). *KIT* mutational analysis of ascites also showed the presence of the *KIT* D816V mutation. Treatment of ASM with midostaurin was started but could not stop progression of ASM with pancytopenia and symptomatic ascites as well as persistent rectal bleeding. Therefore, cytoreductive therapy with cladribine and palliative radiation of the rectal adenocarcinoma was started, which resulted in initial improvement of fatigue, weight loss, bleeding events associated with normalization of hemoglobin, platelets, alkaline phosphatase, and a decrease of the elevated serum tryptase (Figure E1, available in this article's Online Repository at www.jaci-inpractice.org). Of note, response to treatment was also associated with a significant decrease of neoplastic MCs in ascites (Figure 1). The patient then relapsed and died 14 months later.

The second patient was a 62-year-old man presenting with weight loss, recurrent nose bleeding, diarrhea, hepatosplenomegaly, and ascites for 6 months (Table I). Diagnostic workup revealed the diagnosis of SM. In addition, we observed significant anemia, thrombocytopenia, and monocytosis in peripheral blood as well as increased and dysplastic myelopoiesis in BM, resulting in the final diagnosis of SM-AHN, with chronic myelomonocytic leukemia (ASM-CMML-0). Additional NGS of BM could detect mutations in ASXL1, TET2, and STSF2. We again ruled out significant heart and liver diseases as well as significant albuminuria as causes for the ascites, although the patient also suffered from chronic renal disease. Analysis of the ascites revealed a transudate as well as CD117-positive MCs expressing CD25 and CD2 (Figure 1). Monocytosis and spindle-shaped MCs were also found in the ascites. Furthermore, the tryptase level in ascites was 105 ng/mL, suggesting an increase in tryptase resulting from the presence of MCs in ascites. Allogenic stem cell transplantation was performed on conditioning with a FLAMSA-RIC protocol. Unfortunately, SM-AHN persisted after transplantation and was progressive over 12 months. Midostaurin treatment had no significant effect on disease progression and was also associated with side effects. Cutaneous graft-versus-host reaction occurred and had to be treated with tacrolimus and prednisone. The patient is currently in evaluation for a second allogenic transplantation.

In summary, we report 2 patients with advSM and ascites characterized by the presence of neoplastic MCs expressing CD25 and CD2. As MCs develop from pluripotent precursor cells and usually leave the BM for further tissue-specific differentiation under physiologic conditions, the histopathologic pattern of aggregates of MCs infiltrating the BM serves as a major criterion to diagnose SM.[1,2] Although similar aggregates in other extracutaneous organs such as gastrointestinal tract and liver are valued equally as a major diagnostic criterion, the specificity and sensitivity are still a point of discussion.[3] Only few

TABLE I. Characteristics of the 2 patients with advSM before therapy

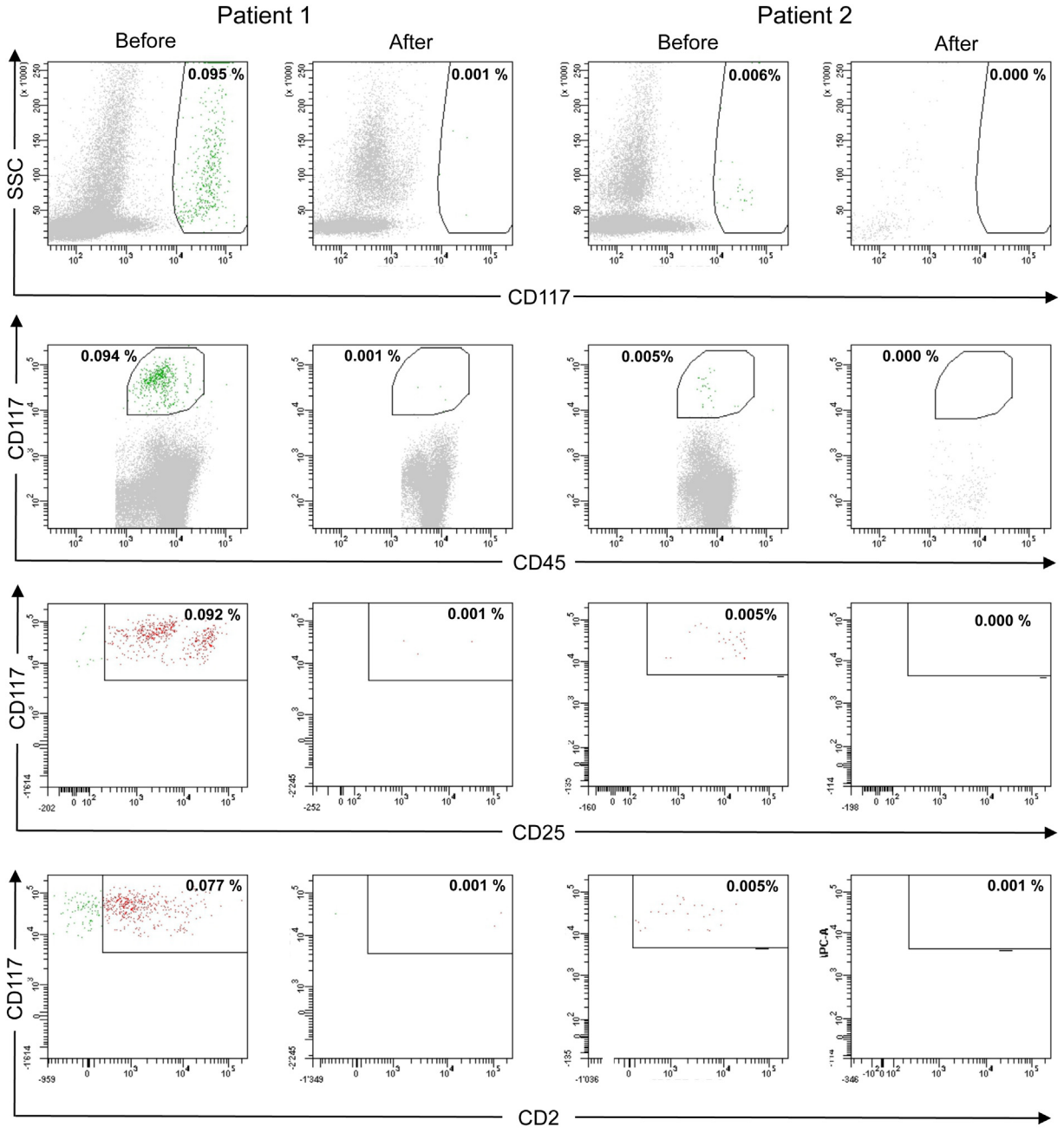
Characteristics	Patient 1	Patient 2
Age at diagnosis (y)	72	62
Sex	Male	Male
Category of mastocytosis	ASM	SM-AHN (ASM-CMML-0)
Diagnostic criteria of SM		
Major criterion		
Multifocal dense infiltrates of MCs in BM	+	+
Minor criteria		
Spindle-shaped MCs in BM, >25%	+	+
CD25/CD2 expression on MCs in BM	+/+	+/+
KIT D816V mutation in BM	+	+
Tryptase level in serum, >20 ng/mL	+ (171 ng/mL)	+ (125 ng/mL)
C-findings		
Cytopenia		
Hemoglobin <10 g/dL	+ (7.5 g/dL)	+ (6.7 g/dL)
Platelets, <100,000 n/mm^3	+ (53,000 n/mm^3)	+ (46,000 n/mm^3)
Neutrophils, <1000 n/mm^3	−	−
Hepatomegaly with ascites	+	+
Splenomegaly with hypersplenism	NK	NK
Malabsorption with weight loss	+	+
Large-sized osteolysis with pathologic fractures	−	−
Life-threatening organ damage caused by MC infiltration	−	−
Additional neoplastic disease	Rectal cancer	−
Additional pathological laboratory findings		
Monocytosis, >620 n/mm^3	−	+ (1560 n/mm^3)
Elevated alkaline phosphatase, >130 U/L	+ (403 U/L)	+ (144 U/L)
Characteristics of ascites		
CD25 expression on MCs in ascites	+	+
CD2 expression on MCs in ascites	+	+
KIT D816V mutation in ascites	+	ND
Tryptase level in ascites (ng/mL)	ND	105 ng/mL
Additional mutations in the BM	SRSF2, TET2, RUNX1	STSF2, ASXL 1, TET 2

"+" indicates presence and "−" absence of characteristic.
advSM, Advanced SM; *ASM*, aggressive SM; *BM*, bone marrow; *CMML*, chronic myelomonocytic leukemia; *MC*, mast cells; *ND*, not determined; *NK*, not known; *SM*, systemic mastocytosis; *SM-AHN*, SM with associated hematological neoplasm.

studies investigated ascites in SM, reporting on lymphocytic hypoalbuminemic ascites associated with few MCs.[4,5] In the present study, we report as a novel finding the presence of neoplastic MCs in ascites in patients with advSM, characterized by expression of CD25 and CD2. Thus, these ascites MCs closely resembled MCs in BM in SM.

Along with these findings, we also detected the *KIT* D816V mutation and a tryptase level of 105 ng/L in ascites. Measurement of tryptase in serum is well established, whereas we could only find few reports in the literature on measurement of tryptase in peritoneal fluid. Berdún et al[6] showed a baseline tryptase level of 0.12 ng/mL with an increase to 4.99 ng/mL after colectomy. We hypothesize that the level of 105 ng/L in our second patient could be indicative of mastocytosis and correlate with MC burden, similar to what has, for example, been shown for tryptase levels in BM.[7] To verify this hypothesis, however, further studies and larger patient populations are needed.

Taken together, we were able to demonstrate the presence of neoplastic MCs in ascites in 2 patients with advSM. In both patients, these MCs were characterized by high expression of CD25 and CD2. Under treatment, the number of CD25- and CD2-expressing ascites MCs decreased. These observations suggest that ascites MCs and possibly also tryptase levels in ascites might serve as additional diagnostic and treatment response markers in patients with advSM when paracentesis is performed for treatment of ascites. However, systematic studies on the presence of MCs in the peritoneal cavity and their exact numbers are sparse, and further functional studies are needed to define whether neoplastic MCs are always present in advSM with ascites or whether they are just "bystanders" derived possibly also from other tissues such as lymph nodes or serosal MCs from peritoneal lining. In the future, it would also be interesting to explore whether isolation and analysis of ascites MCs, as, for example, established for lung MCs,[8] could represent a novel approach to



	$CD117^{bright}$	$CD117^{bright}CD45^{+}$	$CD117^{bright}CD25^{+}$	$CD117^{bright}CD2^{+}$
Before	476	470	460	386
After	4	3	3	2

	$CD117^{bright}$	$CD117^{bright}CD45^{+}$	$CD117^{bright}CD25^{+}$	$CD117^{bright}CD2^{+}$
Before	28	27	27	26
After	0	0	0	0

FIGURE 1. Flow cytometric analysis of mast cells in ascites before and after therapy in the 2 patients with advanced systemic mastocytosis. After doublet elimination and selection for $CD45^{+}$, cells were analyzed for $CD117^{bright}$, $CD117^{bright}CD45^{+}$, $CD117^{bright}CD25^{+}$, and $CD117^{bright}CD2^{+}$ (top to bottom). Percentages of gated cells from all events are shown. The table below shows the number of events for each gate before and after therapy.

evaluate *in vitro* individual diagnostic features and therapeutic responses in advSM.[9]

Acknowledgments

M. Kirsch, G. T. Stehle, J. Passweg, and K. Hartmann provided clinical care; M. Kirsch, G. T. Stehle, J. Passweg, S. C. Meyer, and K. Hartmann conceptualized the study; M. Kirsch, G. T. Stehle, J. Passweg, M. Konantz, S. Dirnhofer, S. C. Meyer, and K. Hartmann performed experiments and/or analyzed data and/or visualized data; M. Kirsch, G. T. Stehle, M. Konantz, S. C. Meyer, and K. Hartmann prepared the original draft of the manuscript; all authors edited and reviewed the manuscript.

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No funding was received for this work.

Conflicts of interest: K. Hartmann has received research funding from Thermo Fisher and consultancy or lecture fees from Allergopharma, Germany, ALK-Abello, Blueprint, Deciphera, Leo Pharma, Menarini, Novartis, Switzerland, Pfizer, United States, Takeda, and Thermo Fisher. S. C. Meyer has consulted for and received honoraria from Celgene/BMS and Novartis. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication March 24, 2022; revised June 28, 2022; accepted for publication July 13, 2022.

Available online July 22, 2022.

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2213-2198

https://doi.org/10.1016/j.jaip.2022.07.014

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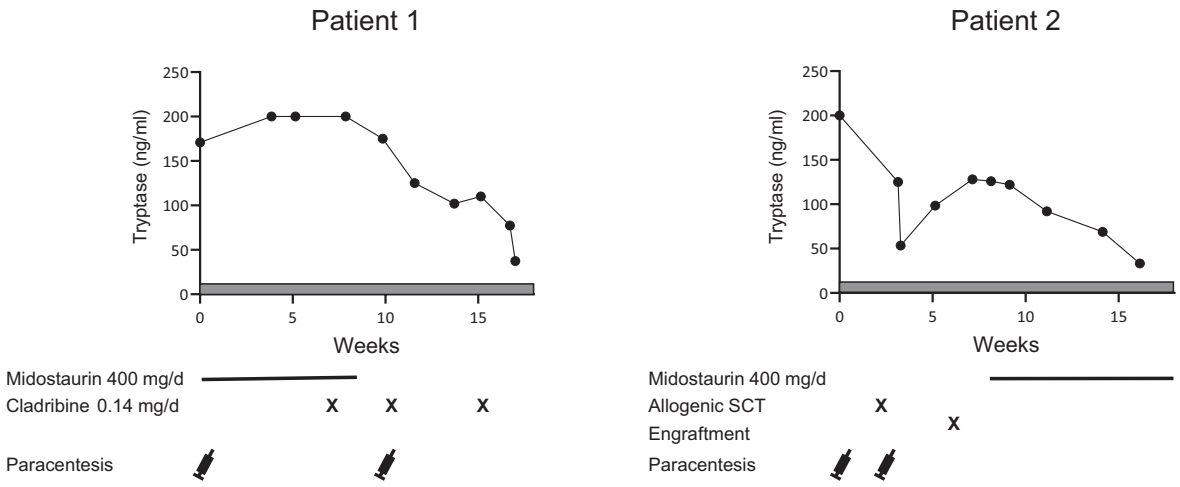

FIGURE E1. Course of serum tryptase levels upon therapy in the 2 patients with advanced systemic mastocytosis. Tryptase levels in the serum over several weeks after the start of therapy. The shaded areas represent the normal range (<11.4 ng/mL). Time course of treatment with midostaurin (daily administration; black line), cladribine (X), bone marrow transplantation and engraftment (X), and paracentesis (black syringe). *SCT,* Stem cell transplantation.