Flow-mediated dilation shows impaired endothelial function in patients with mastocytosis

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Background: Mastocytosis is a rare disease characterized by clonal proliferation of mast cells (MCs) in different organs. Clinical manifestations of mastocytosis are mostly due to release of mediators from MCs and, in many cases, such as urticaria, flushing, angioedema, and anaphylaxis, are an expression of the biological effects of mediators on endothelial cells. Chronic secretion of mediators in patients with mastocytosis can lead to alteration of endothelial function.

Objective: We sought to investigate endothelial function in patients with mastocytosis using a noninvasive technique of flow-mediated dilation (FMD).

Methods: Twenty-five adult patients with indolent and advanced forms of mastocytosis and 20 healthy control subjects were enrolled in the study. Ultrasound assessment of FMD was performed by measuring changes in the diameter of the brachial artery after 5 minutes of arterial occlusion. Changes in FMD were correlated with clinical parameters and serum tryptase levels.

Results: Patients with mastocytosis had lower FMD compared with healthy control subjects ($P < .001$). Advanced and smoldering forms showed a lower FMD compared with indolent forms ($P < .001$). FMD inversely correlated with age and serum tryptase levels and directly with median arterial pressure and recurrent flushing episodes. No correlation was found between FMD and osteoporosis, recurrent anaphylaxis, presence of skin lesions, and long-term antihistamine treatment.

Conclusions: Endothelial dysfunction, as demonstrated by FMD reduction, is detectable in patients with mastocytosis and is more severe in patients with high tryptase levels and advanced disease. Endothelial function appears to be negatively influenced by MC proliferation rather than by the severity of mediator-related symptoms. (J Allergy Clin Immunol 2019;■■■:■■■-■■■.)

Key words: *Endothelial cells, endothelial function, flow-mediated dilation, mast cells, mastocytosis, mediators, tryptase*

Abbreviations used

CysLT:	Cysteinyl leukotriene
EC:	Endothelial cell
FMD:	Flow-mediated dilation
MAP:	Mean arterial pressure
MC:	Mast cell
NO:	Nitric oxide
PAF:	Platelet-activating factor
PGD_2:	Prostaglandin D_2
ROS:	Reactive oxygen species
SM:	Systemic mastocytosis
SMC:	Vascular smooth muscle cell

Mastocytosis is a clinically heterogeneous disease characterized by clonal proliferation of mast cells (MCs) in various tissues and organs, including the skin, bone marrow, gastrointestinal tract, liver, spleen, and lymph nodes.[1] Adults usually have the systemic form of the disease and show skin lesions and/or involvement of at least an extracutaneous organ, most frequently the bone marrow.[2]

The current classification defines systemic mastocytosis (SM) as a complex disease with multiple variants ranging from indolent forms, with life expectancy similar to that of the general population, to advanced forms, which are associated with poor prognosis.[3] Clinical signs and symptoms of patients with mastocytosis are primarily caused by release of MC-derived proinflammatory and vasoactive mediators and organ dysfunction caused by progressive MC infiltration. Although the latter are usually restricted to patients with advanced forms of the disease, symptoms caused by MC mediator release are seen in patients with all variants of mastocytosis and are independent from the rate of MC proliferation.[4,5] Mediators released by MCs include histamine; lipid mediators, such as prostaglandin D_2 (PGD_2), cysteinyl leukotrienes (CysLTs), and platelet-activating factor (PAF); heparin; serotonin; tryptase; and multiple cytokines and chemokines.[6,7] Mediator-related symptoms are present in the majority of patients with mastocytosis, and they are attributed to an uncontrolled, acute, or chronic secretion of MC mediators.

Symptoms caused by MC mediators can be cutaneous (urticaria, pruritus, flushing, and angioedema), gastrointestinal (abdominal pain, gastritis, and diarrhea), musculoskeletal (bone pain and osteoporosis), and cardiovascular (hypotension, syncope, or recurrent anaphylaxis).[8-10] Many of these symptoms are caused by multiple effects of MC mediators on the local (urticaria, angioedema, and diarrhea) or systemic (hypotension and anaphylaxis) microcirculation. It is well known that MC-derived mediators profoundly influence vascular tone and blood flow by inducing vasodilation and increasing vascular

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permeability. These effects are mainly due to the interaction of mediators with endothelial cells (ECs) and vascular smooth muscle cells (SMCs). The effects of mediators, such as histamine, PGD_2, CysLTs, and PAF, on EC and SMC functions have been fully elucidated over the last decades.[11] These mediators have pleotropic and redundant vasoactive proprieties, including activation of ECs and relaxation of SMCs. Together, these effects result in increased blood flow, reduced vascular resistance, and increased endothelial leakage.

Despite plenty of evidence on the effects of MC mediators on vascular function, endothelial responses *in vivo* have not been investigated thus far in patients with SM. Flow-mediated dilation (FMD) is a widely used technique to study endothelial function in patients with several cardiovascular diseases. It is a noninvasive method that measures flow-mediated changes in brachial artery diameter induced by short-term ischemia.[12] Flow-mediated changes are caused by stress-induced generation of endothelium-derived vasoactive mediators. Because FMD is almost completely blocked by pretreatment with nitric oxide (NO) synthase inhibitors, it has been proposed that this response is mainly due to endothelial release of NO.[13] MCs secrete a variety of mediators that potentially interfere with generation of NO by ECs and therefore can modulate endothelial response to ischemic stress. The aim of this study has been to investigate endothelial function in adult patients with SM by using FMD.

METHODS

Patient selection and data collection

In this cross-sectional study we included 25 patients with SM referred to the Mastocytosis Reference Centre of the University of Salerno. Patients with SM were classified according to World Health Organization criteria revised by Valent et al.[2] Eighteen (72%) patients had indolent SM, 2 (8%) patients had smoldering SM, 4 (16%) patients had aggressive SM, and 1 (4%) patient had SM associated with another hematologic disorder. KIT mutation analysis was performed in 8 (32%) patients, and all of them carried the D816V mutation.

As a control group, we enrolled 20 healthy subjects matched for age, sex, body mass index, and smoking status with the patient group. A previous history of cancer, myocardial infarction, venous thromboembolism, stroke, diabetes, arterial hypertension, or heart failure was an exclusion criterion for both patients and control subjects. Clinical history, anthropometric data, and vital signs were collected immediately before FMD evaluation. Demographic and clinical characteristics of control subjects and patients are reported in Table I. Grading of mediator-related symptoms was determined according to Valent et al.[14]

A subset of patients analyzed in this study received continuous antihistamine treatment defined as daily intake of an oral antihistamine at the recommended dosage for at least 3 months before FMD evaluation. In addition to antihistamines, additional antimediator drugs, including H_2 blockers and antileukotrienes, were given to 2 patients. At the time of FMD evaluation, no patient was receiving treatment with glucocorticoids, nonsteroidal anti-inflammatory drugs, or supplements (vitamin C, vitamin E, or N-acetylcysteine) that could influence FMD. Four patients with aggressive mastocytosis were treated with cytoreductive drugs (2 with cladribine and 2 with midostaurin). However, FMD evaluation was performed in all patients before the beginning of these treatments.

The study protocol was approved by the local ethics committee and conducted in accordance with the principles embodied in the Declaration of Helsinki.[15] Written informed consent was obtained from all enrolled subjects.

FMD

Ultrasound assessment of endothelial-dependent FMD was made according to the guidelines of the American College of Cardiology.[16] FMD was performed in a temperature-controlled room (22°C) with subjects in a resting supine state between 8 and 10 AM after at least an 8-hour fast. Brachial artery diameter was imaged by using a 7.5-MHz linear array transducer ultrasound system (Toshiba, Minato, Tokyo) equipped with electronic calipers, vascular software for 2-dimensional imaging, color and spectral doppler imaging, and internal electrocardiography. The brachial artery was visualized 3 to 7 cm above the antecubital crease. To create a flow stimulus, a sphygmomanometer cuff was placed on the forearm, and the cuff was inflated at least 50 mm Hg greater than systolic pressure to occlude artery inflow for 5 minutes. At the end of 5 minutes of ischemia, brachial artery diameter was measured above the cuff within 1 minute after releasing occlusion. Measurement within 1 minute after ischemia allows an optimal assessment of FMD. All vasodilatation measurements were synchronized at the beginning of the electrocardiographic R wave taken at the end of diastole. FMD was expressed as the change in poststimulus diameter evaluated as the percentage increase from the baseline diameter. FMD was performed by the same operator in all patients to ensure consistency.

TABLE I. Demographic and clinical characteristics of control subjects and patients

	Control subjects (n = 20)	Patients with mastocytosis (n = 25)	*P* value
Age (y), range	43.8 ± 14, 24-67	45.3 ± 12, 24-65	.213
Female sex, no. (%)	11 (61)	16 (64)	.578
Smokers, no. (%)	4 (20)	5 (20)	.712
Body mass index (kg/m^2)	24.4 ± 3.7	24.8 ± 3.1	.581
Systolic blood pressure (mm Hg)	122.7 ± 10.6	118.4 ± 15.7	.466
Diastolic blood pressure (mm Hg)	76.3 ± 7.4	71.6 ± 8.9	.183
MAP (mm Hg)	91.8 ± 6.8	87.2 ± 9.0	.146
Heart rate (beats/min)	70.3 ± 5.2	73 ± 8.2	.547
Hemoglobin (g/dL)	13.1 ± 1.7	12.9 ± 1.6	.689

Data are expressed as means ± SDs. Values in parentheses indicate percentages of patients.

Serum tryptase assay

Serum tryptase levels were measured from blood samples drawn by means of venipuncture after at least 8 hours of fasting by using an immune enzymatic method (ImmunoCAP; Thermo Fisher, Waltham, Mass). In patients with recurrent anaphylaxis, serum tryptase levels were measured at least 2 weeks after the acute episode.

Statistical analysis

Categoric variables were reported as counts (percentages), and continuous variables were reported as means ± SDs or medians and interquartile ranges, when appropriate. Categoric variables were compared by using χ^2 tests. Normal distribution of parameters was assessed by using the Kolmogorov-Smirnov test. The Student unpaired *t* test, Mann-Whitney *U* test, and ANOVA with Bonferroni *post hoc* analysis were used to compare continuous variables.

Univariate and multivariable linear regression analyses were performed to explore correlations between FMD and clinical or laboratory parameters in patients with mastocytosis. In all analyses a *P* value of less than .05 was considered statistically significant. A multivariable linear regression analysis was performed by using variables with a *P* value of less than .05 at univariate analysis. Sample size calculation was computed with respect to a 2-tailed Student *t* test for independent groups, considering 4% (δ) as the difference for FMD between patients and control subjects, 3% as the SD, 0.05 (α) as the type I error probability, and 0.90 as power 1-β. The sample size was 26 patients, with 13 patients for each group. All tests were 2-tailed, and analyses were performed with computer software packages (IBM SPSS Statistics, version 22.0; IBM, Armonk, NY).

TABLE II. Clinical characteristics of patients with mastocytosis

All patients, no. (%)	25 (100)
Patients with skin lesions (urticaria pigmentosa), no. (%)	17 (68)
Mediator-related symptoms, no. (%)	
Osteoporosis	16 (64)
Pruritus	12 (48)
Flushing	9 (36)
Diarrhea	8 (32)
Urticaria	7 (28)
Anaphylaxis	6 (24)
Continuous antihistamine treatment, no. (%)	10 (40)
Tryptase (μg/L)	
Median	47.4
Interquartile range	27.1-135.5

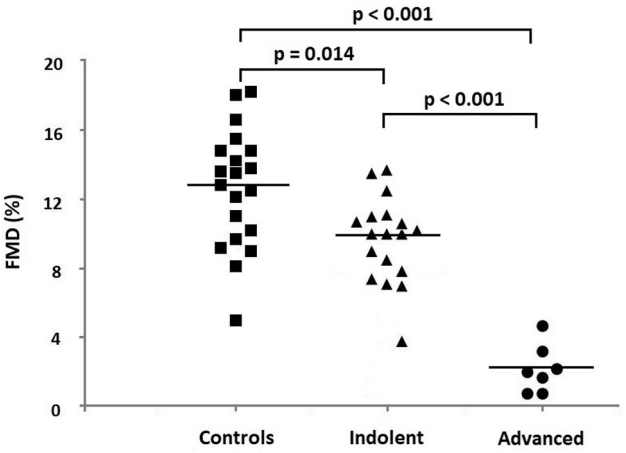

FIG 1. FMD values in control subjects and patients with mastocytosis. FMD values were measured as described in the Methods section in 20 healthy subjects *(controls)*, 18 patients with indolent SM, and 7 patients with advanced SM. FMD is expressed as percentage variation in brachial artery diameter induced by 5 minutes of ischemia. *Lines* indicate mean values in each group.

RESULTS

Demographic and clinical characteristics of control subjects and patients are shown in Table I. There was no significant difference in age, sex, smoking status, and clinical parameters relevant to FMD between the 2 groups. Table II shows the clinical characteristics of patients with mastocytosis. All patients underwent FMD evaluation, as described in the Methods section. Basal diameter of the brachial artery at the beginning of the procedure was not significantly different between patients and control subjects (3.47 ± 0.67 vs 3.43 ± 0.49 mm, $P = .854$). Patients with mastocytosis had a significantly lower FMD compared with control subjects (7.6% ± 3.9% vs 13.0% ± 4.5%, $P < .001$). FMD was significantly reduced in both patients with indolent and advanced forms of the disease compared with that in control subjects (Fig 1). Interestingly, however, the severity of the disease had a major effect on FMD because patients with advanced forms had a significantly lower FMD compared with patients with indolent forms (2.1% ± 1.1% vs 8.4% ± 3.1%, $P < .001$). For this analysis, patients with smoldering mastocytosis were included in the group with advanced form of the disease.

The serum tryptase level is a surrogate marker of MC proliferation in patients with mastocytosis and is a negative prognostic factor of the disease. Therefore we investigated the relationship between FMD and tryptase levels in patients with mastocytosis. Fig 2, *A*, shows that there is a significant negative correlation between FMD and serum tryptase level ($P < .001$), indicating that severe endothelial dysfunction is detectable in patients with high tryptase levels. The inverse correlation between FMD and tryptase levels remains highly significant in the subgroup of patients with indolent (Fig 2, *B*) and advanced (Fig 2, *C*) mastocytosis.

Univariate analysis showed that, in addition to tryptase level, FMD correlated negatively with age and recurrent flushing and positively with mean arterial pressure (MAP; Table III). By contrast, sex, body mass index, and smoking status showed no correlation with FMD. Furthermore, no correlation was found between FMD and osteoporosis, recurrent anaphylaxis, and the presence of skin lesions. Multivariate regression analysis was further performed for variables that were significant at univariate analysis. This analysis confirmed that tryptase level, age, and flushing were strong independent variables associated with FMD reduction (Table III). Interestingly, Fig 3, *A*, shows that there is a highly significant negative correlation between FMD and age ($P = .002$). The inverse correlation between FMD and age remains significant in the subgroup of patients with indolent (Fig 3, *B*) but not those with advanced mastocytosis (Fig 3, *C*).

Histamine is a powerful vasoactive mediator released from MCs causing endothelium-dependent vasodilation through activation of H_1 receptors. To understand whether histamine was involved in causing FMD changes in patients with mastocytosis, we compared FMD in patients receiving long-term antihistamine treatment with that in untreated patients. FMD was not significantly different in patients with or without continuous antihistamine treatment (8.0% ± 3.6% and 7.0% ± 4.5%, respectively; $P = .565$; Table III).

Finally, to explore whether there was any relationship between the severity of mediator-related symptoms and FMD, we compared endothelial function in patients with different symptom grading. Symptom grading was scored according to Valent et al,[14] with 0 defined as no symptoms and 3 defined as severe symptoms. There was no significant difference in FMD values between patients with grade 0 (FMD: 7.3% ± 3.3%), grade 1 (FMD: 9.5% ± 4.2%), grade 2 (FMD: 7.0% ± 4.8%) and grade 3 (FMD: 7.4% ± 0.4%; $P = .717$).

DISCUSSION

Noninvasive assessment of endothelial function by FMD reveals reduced endothelium-mediated vasodilation in adults with mastocytosis. This study provides the first demonstration of the impairment of endothelial function in patients with SM in comparison with healthy control subjects. Impairment of FMD is greater in patients with advanced forms of mastocytosis compared with those with indolent SM.

FMD is a widely used technique to assess endothelial dysfunction defined as any form of functional and reversible alteration leading to an abnormal endothelial response to physiologic stimuli. Endothelial function is then shifted toward reduced vasodilation and acquisition of a proinflammatory, proliferative, and prothrombotic state.[17] It should be mentioned that the FMD technique does not fully discriminate between endothelial and SMC dysfunction. FMD evaluation should have

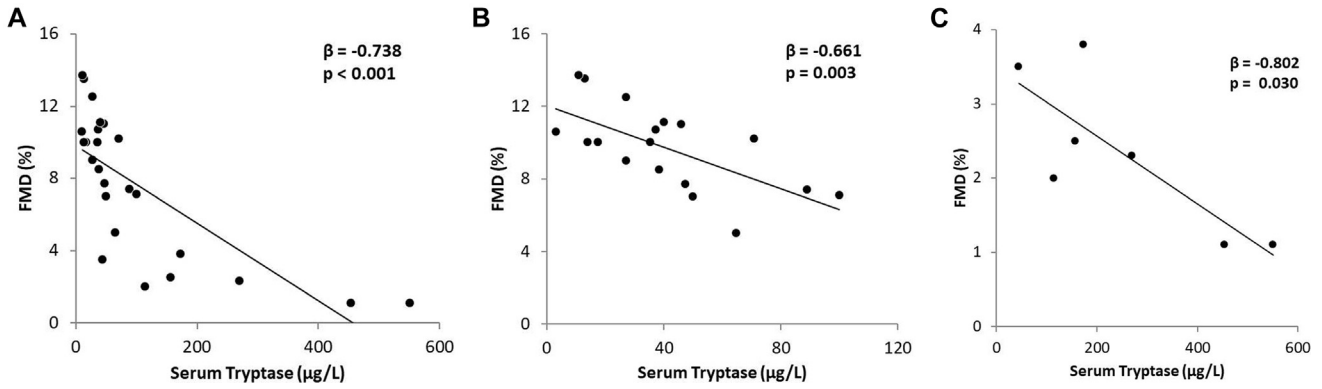

FIG 2. Correlation between FMD and serum tryptase levels in the whole population of patients with mastocytosis **(A)** and in patients with indolent **(B)** and advanced **(C)** forms of the disease. FMD was measured as described in the Methods section and expressed as percentage variation in brachial artery diameter induced by 5 minutes of ischemia. Serum tryptase levels were measured by using an immunoenzymatic method.

been done after administration of nitroglycerin, which causes an endothelium-independent relaxation, to analyze endothelial and SMC dysfunction separately. However, we decided to avoid this test because it can cause severe hypotension.[16] FMD is altered in patients with atherosclerosis, diabetes, hypertension, and dyslipidemia.[18] Several studies indicate that FMD provides independent prognostic information that exceeds the predictive value of traditional risk factors. This parameter appears to predict cardiovascular death and cardiovascular events, such as myocardial infarction, stroke, heart failure, and peripheral artery disease.[19,20]

Several mechanisms can be hypothesized as causes of the reduced FMD seen in patients with mastocytosis. First, the reduced response might be related to persistent vasodilation induced by chronic release of MC-derived vasoactive mediators. It is tempting to speculate that continuous release of MC mediators maintains a stable peripheral vasodilation that precludes the increase in blood flow during FMD. Mediators inducing vasodilation that are continuously secreted in patients with mastocytosis include histamine, PGD_2, CysLTs, and PAF. Histamine induces vasodilation and increases vascular permeability through H_1 receptors that, in turn, activate the Akt1 pathway, leading to NO production.[11] In addition, it has been demonstrated that histamine can cause H_1 receptor desensitization,[21] and therefore it can be hypothesized that, at the time of FMD, peripheral vessels might be unresponsive to the vasodilatory action of histamine. Urinary levels of histamine metabolites are consistently increased in patients with mastocytosis, and these levels are correlated with serum tryptase.[22] The latter observation is in line with our finding that FMD reduction correlates with serum tryptase levels.

Our data show that continuous antihistamine treatment does not influence FMD, thereby arguing against a role of histamine causing chronic H_1 receptor activation, desensitization, or both, as the main cause of impaired FMD in patients with mastocytosis. It should be mentioned that the subgroup analysis in patients with or without continuous antihistamine treatment was performed with a limited number of cases, and therefore further studies on a larger population sample are required to confirm our conclusions. Other vasoactive mediators secreted by MCs, such as CysLTs, PGD_2, and PAF, can induce persistent vasodilation independently from histamine.[23]

TABLE III. Univariate and multivariate regression analysis of factors correlated with FMD in patients with mastocytosis

	Univariate analysis		Multivariate analysis	
	β	*P* value	β	*P* value
Age	−0.590	.002	−0.419	.004
Female sex	0.372	.067	NS	NS
Body mass index	−0.260	.210		
MAP	0.533	.006	NS	NS
Smoking	−0.279	.177		
Patients with skin lesions (urticaria pigmentosa)	−0.216	.322		
Osteoporosis	−0.058	.781		
Pruritus	−0.069	.744		
Flushing	−0.428	.033	−0.329	.013
Diarrhea	−0.111	.597		
Urticaria	−0.045	.830		
Anaphylaxis	−0.152	.880		
Continuous antihistamine treatment	−0.121	.565		
Tryptase	−0.738	<.001	−0.598	.002

Multivariable linear regression analysis was performed by using variables with a *P* value of less than .05 at univariate analysis. β Values are defined as the correlation coefficient.
NS, Not significant.

In addition, tryptase might be involved in endothelial dysfunction by inducing direct activation of protease-activated receptor 2.[24] Activation of protease-activated receptor 2 on ECs induces leukocyte rolling and adhesion, NO impairment, increase in vascular permeability, and overexpression of COX-2.[25-27] Together, these alterations can induce a procoagulant and proinflammatory state that could further contribute to generate endothelial dysfunction in mastocytosis.

A second explanation for reduced FMD in mastocytosis is an increased arterial stiffness related to MC infiltration of the arteriolar wall. Several studies have shown that in both healthy subjects and patients with many pathologic conditions, MCs are located around blood vessels and can infiltrate all layers of the arterial wall.[28] Studies in animal models demonstrate that MCs are abundant in close proximity of arterioles.[29] MC infiltration of precapillary arterioles might increase vessel stiffness and

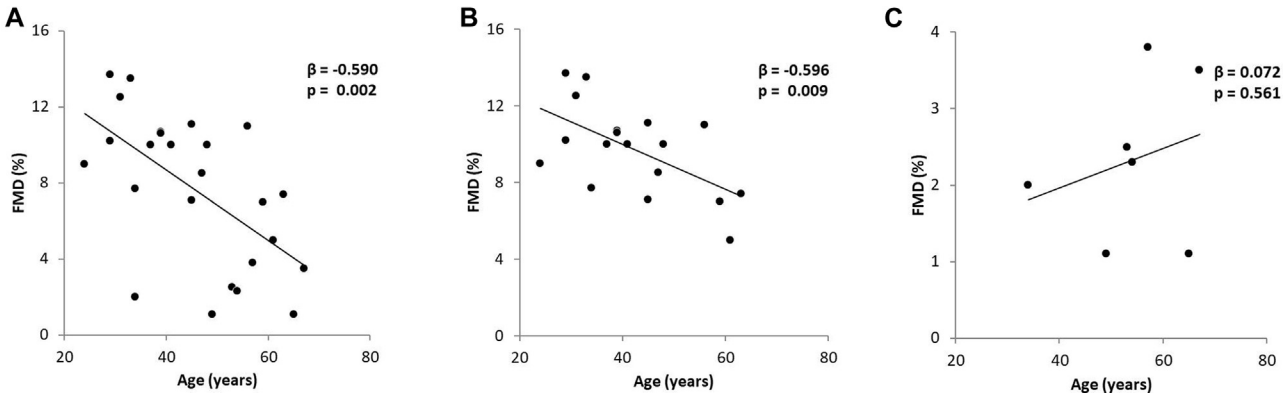

FIG 3. Correlation between FMD and age in the whole population of patients with mastocytosis **(A)** and in patients with indolent **(B)** and advanced **(C)** forms of the disease. FMD was measured and expressed as percentage variation in brachial artery diameter induced by 5 minutes of ischemia.

contribute to the reduced FMD detected in patients with mastocytosis. The observation that FMD is inversely related to serum tryptase level, a reliable indicator of MC proliferation, supports this hypothesis.

Finally, a third mechanism by which FMD can be altered in patients with mastocytosis is enhanced oxidative stress associated with MC proliferation causing an impairment in NO production by ECs.[30] Recent evidence demonstrates high serum levels of 2 markers of oxidative stress (advanced glycation end-products and advanced oxidation protein products) in patients with SM.[31] MC activation and proliferation are associated with enhanced reactive oxygen species (ROS) production mainly by using NADPH oxidase 2.[32,33] Furthermore, ROS play an important role in SM progression because they induce expression of the antiapoptotic protein DJ-1 in clonal MCs[34] ROS generation associated with MC proliferation could impair endothelium-mediated dilation through several mechanisms, including (1) inhibition of vasodilator prostacyclin production, (2) uncoupling endothelial NO synthase, and (3) binding of NO with subsequent reduction of its bioavailability.[35]

In patients with SM, univariate analysis shows that age, high serum tryptase levels, and flushing are associated with worse FMD, whereas MAP is directly related to FMD. The correlation between FMD and age was previously demonstrated by several studies showing that aging is associated with a progressive reduction of vascular compliance.[36] Interestingly, the negative correlation between age and FMD is significant in the subgroup of patients with indolent mastocytosis but not in patients with advanced disease. These data indicate that in patients with severe forms of mastocytosis, the effect of the disease on endothelial function overcomes that of physiologic aging of blood vessels and strongly points to factors associated with the disease as the primary cause of FMD alterations in patients with mastocytosis. The presence of flushing is associated with reduced FMD, probably as an expression of altered vasomotor tone induced by PGD_2. It is interesting to note that a reduced FMD has been reported in patients with postmenopausal flushing.[37]

An intriguing finding of our study is that FMD is reduced in patients with the lowest MAP. These data might be in contrast to the well-known observation that reduced FMD is associated with hypertension. However, because hypertensive patients were excluded from our study, our finding suggests that in patients with mastocytosis, low blood pressure, which is often detected in patients with advanced forms of the disease, is associated with impaired vasodilation.

It is known that FMD impairment is associated with a high risk of major cardiovascular events.[38] The cardiovascular risk has not been fully evaluated in patients with mastocytosis. Two studies suggest that these patients might be at higher risk for certain cardiovascular events.[39,40] More conclusive evidence of the cardiovascular risk in patients with mastocytosis are highly desirable, and whether the reduction in FMD could be a predictive factor will be investigated in further long-term studies.

In conclusion, our data show that patients with mastocytosis have endothelial dysfunction, as indicated by a lower FMD compared with that seen in healthy control subjects. FMD impairment is more evident in advanced forms of the disease and appears to be correlated with MC burden. Further studies are needed to understand the mechanisms underlying endothelial dysfunction in patients with mastocytosis and to define its clinical and prognostic effect on the disease.

Clinical implications: Endothelial function is impaired in patients with mastocytosis, particularly those with high tryptase levels, and might contribute to an increased cardiovascular risk and poor prognosis of advanced forms.

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