

## Letter to the Editor

**Serum IL-31 levels are increased in a subset of patients with mastocytosis and correlate with disease severity in adult patients**

To the Editor:

Mastocytosis is characterized by activating *KIT* mutations and clonal expansion of mast cells (MCs) in tissues, particularly in skin and bone marrow.<sup>1</sup> The expansion of MCs is variable, resulting in a broad spectrum of different disease categories that encompass benign subtypes, such as cutaneous mastocytosis (CM) and indolent systemic mastocytosis (ISM), as well as advanced categories, such as aggressive systemic mastocytosis and MC leukemia.<sup>2</sup> Symptoms of mastocytosis do not always correlate with the extent of MC infiltration and include pruritus, urticaria pigmentosa, anaphylaxis, flushing, headache, diarrhea, and osteoporosis.<sup>3</sup> Benign subtypes are mainly treated by avoidance of MC activation and H<sub>1</sub> antihistamines. Treatment options in advanced categories also comprise IFN- $\alpha$ , cladribine (2-chlorodeoxyadenosine), and tyrosine kinase inhibitors targeting *KIT* activation.

Several biomarkers are known to correlate with MC burden in patients with mastocytosis.<sup>4</sup> The best-characterized marker is the MC-derived enzyme tryptase, which is also used in clinical routine for diagnosis and to monitor patients with mastocytosis. In addition, serum soluble *KIT*, soluble CD25, IL-6, IL-13, carboxypeptidase A, several neuropeptides, and urinary histamine metabolites have been described to correlate with the extent of mastocytosis.

In recent years, it has become evident that the novel cytokine IL-31 plays an important role in the induction of chronic skin inflammation.<sup>5,6</sup> Patients with atopic dermatitis, chronic spontaneous urticaria, allergic contact dermatitis, prurigo nodularis, and primary cutaneous lymphomas express increased serum IL-31 and *IL31* mRNA levels in the skin.<sup>6,7</sup> IL-31 serum levels in patients with atopic dermatitis also correlate with disease activity<sup>7</sup> and levels of T<sub>H</sub>2 cytokines, such as IL-4 and IL-13. Moreover, gene variants of *IL31* are associated with the development of atopic dermatitis. The source of IL-31 are CD4<sup>+</sup> T cells, particularly activated T<sub>H</sub>2 cells, peripheral blood, and skin-homing CD45RO CLA<sup>+</sup> T cells and MCs, as shown recently in the skin of patients with psoriasis.<sup>8</sup> Moreover, monocytes, macrophages, and dendritic cells produce IL-31 on UV irradiation and treatment with hydrogen peroxide. Activated monocytes, macrophages, eosinophils, basophils, dorsal root ganglia, and keratinocytes have been found to express the IL-31 receptor complex.<sup>6</sup>

To our knowledge, the involvement of IL-31 in mastocytosis has not yet been investigated. We therefore aimed to clarify whether IL-31 levels are increased in mastocytosis and whether IL-31 levels differ between disease categories.

Expression of IL-31 was analyzed in sera of 50 patients with different categories of mastocytosis, as classified according to established World Health Organization criteria,<sup>1</sup> and 10 healthy control subjects. Detailed clinical characteristics of all patients are listed in Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). In all patients and control subjects, total serum tryptase levels were measured by using the fluoroimmunoassay (ImmunoCAP Tryptase; Thermo Fisher Scientific, Uppsala, Sweden). Serum IL-31 levels were measured by

using ELISA (BlueGene Biotech, Shanghai, China). The sensitivity limit of the ELISA was less than 1.0 pg/mL, and the detection limit was 1000 pg/mL. Statistical analysis was performed with GraphPad Prism software (version 5.01; GraphPad Software, La Jolla, Calif) by using the nonparametric Mann-Whitney *U* test. Disease categories and bone marrow involvement were also analyzed with Kaplan-Meier curves by using the log-rank test. Correlation analysis was performed with Spearman rank order correlation. A *P* value of less than .05 was considered statistically significant.

For analysis of IL-31 protein expression in cutaneous and bone marrow lesions of patients with mastocytosis, sections of paraffin-embedded skin biopsy specimens of 3 patients and bone marrow biopsy specimens of 10 patients were analyzed by using double-label immunofluorescence with the M.O.M. Immunodetection Kit (Vector Laboratories, Burlingame, Calif). After pretreatment (Vector Antigen Unmasking Solution), sections were stained with mouse anti-human IL-31 mAb (R&D Systems, Wiesbaden, Germany) or MC tryptase mAb (Chemicon, Bedford, Mass) overnight at 4°C, followed by incubation with biotinylated anti-mouse IgG and labeling with Fluorescein Avidin DCS or Texas Red Avidin DCS (Vector Laboratories).

Serum IL-31 levels were significantly increased in patients with mastocytosis compared with those seen in healthy control subjects (*P* < .0473; Fig. 1, A). While control subjects showed a range of IL-31 levels between 23.1 and 111.3 pg/mL, levels in patients with mastocytosis ranged between 24.5 and 781.0 pg/mL (Fig 1, A). Interestingly, within the group of 38 adult patients, those with advanced disease categories, such as smoldering systemic mastocytosis and aggressive systemic mastocytosis with associated clonal hematologic non-MC lineage disease, exhibited significantly increased IL-31 levels compared with those with nonadvanced disease categories, including CM or ISM and ISM with associated clonal hematologic non-MC lineage disease (*P* = .0006 [Fig 1, A] and *P* = .0005 [Fig 1, B]). All IL-31 values of greater than 313.9 pg/mL were measured in patients with advanced disease categories, and none of the patients with advanced disease categories were found to exhibit IL-31 values of less than 81.9 pg/mL (Fig 1, A). As anticipated, tryptase levels increased along with disease severity (see Fig E1, A, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), and consistently, IL-31 was found to also correlate with tryptase in the subgroup of adult patients (*P* = .0093; Fig 1, C). Furthermore, IL-31 levels correlated with the percentage of MC infiltrates in bone marrow (*P* = .0404; see Fig E2, E, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), and patients with MC infiltrates of greater than 15% showed significantly increased IL-31 levels compared with patients with infiltrates of 15% or less (*P* = .0306; see Fig E2, F). Surprisingly, when we compared the group of 12 pediatric patients, who all had CM, with the group of 28 adult patients with CM or ISM, we observed significantly increased IL-31 levels in pediatric patients (*P* = .0279; Fig 1, A). Because pediatric patients typically show lower tryptase levels than adult patients, which was also confirmed in our study (see Fig E1, A), this finding was unexpected.

Analyzing different clinical parameters, we detected significantly increased IL-31 levels in male compared with female patients, which might be due to increased body weight associated

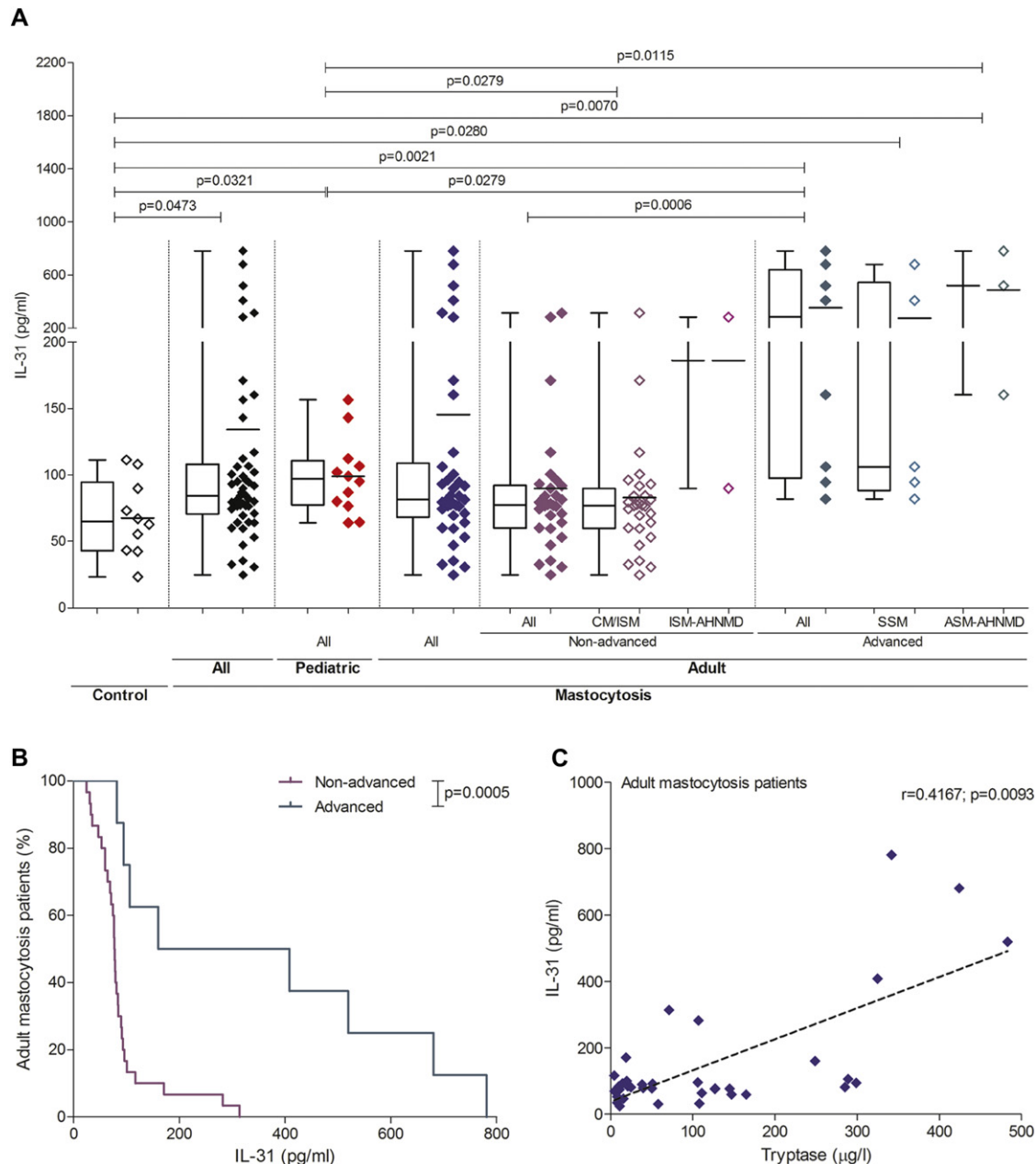


**FIG 1.** A, Serum IL-31 levels are increased in patients with mastocytosis (*All*) compared with those seen in healthy control subjects (*Control*). Adult patients with advanced mastocytosis categories show increased IL-31 levels compared with those with nonadvanced mastocytosis categories. Data are presented as box plots (minimum to maximum) with medians (*left*) and scatter dot plots with means (*right*). B, Adult patients with advanced disease categories show significantly increased IL-31 levels compared with those with non-advanced disease categories when levels were compared by using Kaplan-Meier curves with the log-rank test. C, IL-31 levels (*y*-axis) positively correlate with tryptase serum levels (*x*-axis) in all adult patients with mastocytosis.

with increased MC numbers in male patients ( $P = .0338$ ; see Fig E2, A). There was also a trend toward increased IL-31 expression in patients with osteosclerosis compared with that seen in patients with normal bone density, osteopenia, or osteoporosis, reflecting again the association between advanced categories of mastocytosis and high IL-31 levels because all 3 patients with osteosclerosis had either smoldering systemic mastocytosis ( $n = 1$ ) or ASM-AHNMD ( $n = 2$ ; not significant; see Fig E2, H). A relationship between increased bone density and increased tryptase levels

has already been described.<sup>9</sup> In contrast, other clinical parameters, including age, disease duration, presence of a KitD816V mutation or skin lesions, presence of MC activation symptoms (eg, pruritus, anaphylaxis, flushing, and diarrhea), and intake of  $H_1$  antihistamines, did not affect IL-31 levels (see Fig E2, B, G, and I-P).

To determine the cellular source of IL-31, we next analyzed skin and bone marrow biopsy specimens of patients with mastocytosis by using immunohistochemistry with specific antibodies against tryptase and IL-31 (Fig 2). In both tissues expression of IL-31

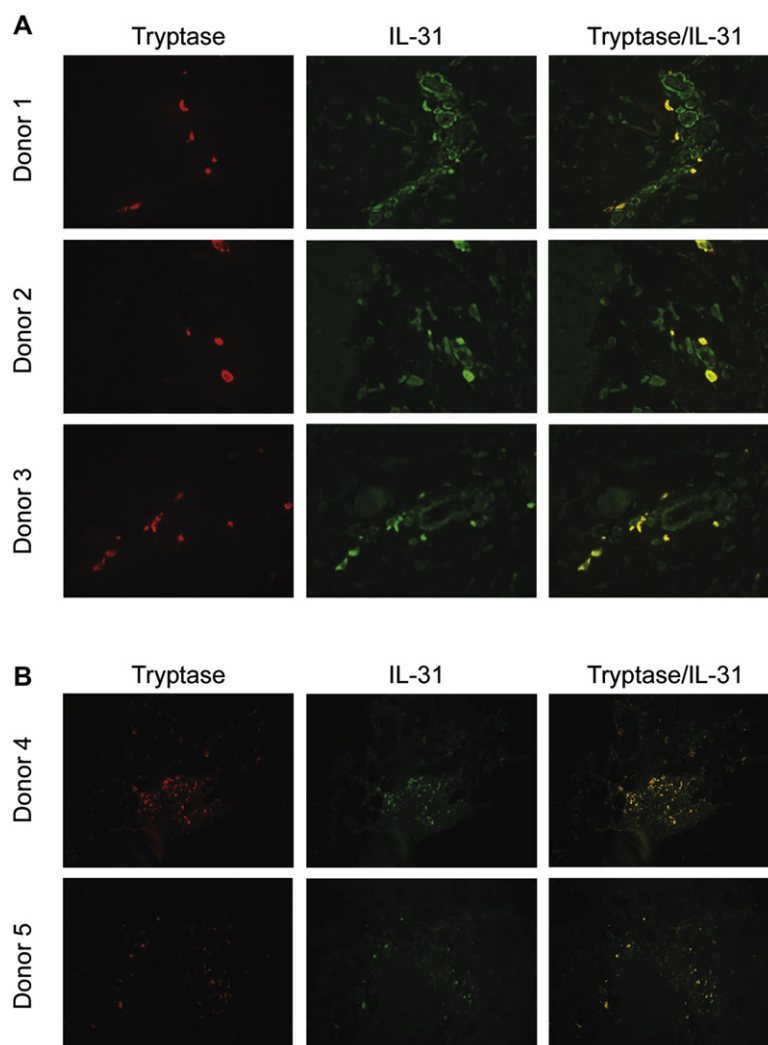


**FIG 2.** Tryptase-positive MCs are the source of IL-31 in skin (A) and bone marrow (B) of patients with mastocytosis. Double immunofluorescence staining of paraffin-embedded skin (Fig 2, A) and bone marrow biopsy specimens (Fig 2, B) was performed with antibodies against tryptase (red) and IL-31 (green), demonstrating colocalization of both antibodies (yellow; magnification  $\times 63$  [Fig 2, A] and  $\times 20$  [Fig 2, B]).

clearly colocalized with tryptase-positive MCs, demonstrating that MCs represent a major source of IL-31 in mastocytosis.

In conclusion, our results show, for the first time, that patients with mastocytosis express increased serum IL-31 levels. In adults, IL-31 levels correlate with disease severity, tryptase levels, and percentages of bone marrow infiltration. MCs in skin and bone marrow of patients with mastocytosis are able to produce IL-31. Our data suggest exploiting IL-31 levels as a potential diagnostic marker in adult patients, particularly in patients with mastocytosis-associated osteosclerosis, which might indicate disease progression. Furthermore, to our knowledge, this is the first report on expression of IL-31 in bone marrow.

We thank C. Berns (Department of Dermatology, University of Cologne, Cologne, Germany) for excellent technical assistance.

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Supported by research grants from the German Research Council (DFG; CRC/SFB832, project A14), the German-Israeli Foundation for Scientific Research and Development (993/2008), and the Wilhelm Sander Foundation (1999.049.2) to (K.H.).

Disclosure of potential conflict of interest: K. Hartmann has consultant arrangements with Novartis and has received payment for lectures from ALK-Abelló, Novartis, and Shire. The rest of the authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2012.11.008>

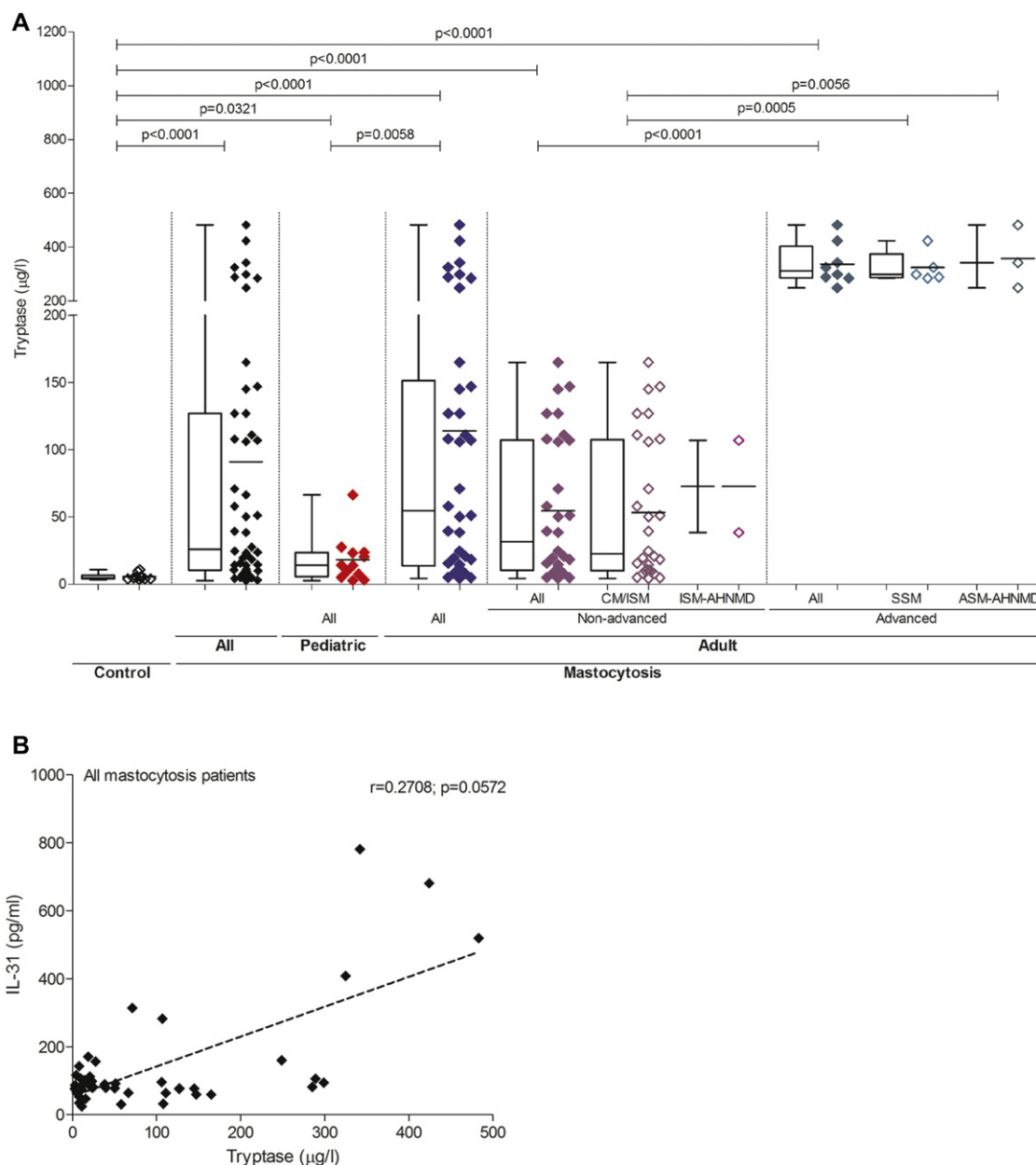


**FIG E1. A**, As expected, serum tryptase levels are significantly increased in patients with mastocytosis (*All*,  $n = 50$ ) compared with those seen in healthy control subjects (*Control*,  $n = 10$ ). Tryptase levels in adult patients (*All*,  $n = 38$ ) are increased compared with those seen in pediatric patients (*All*,  $n = 12$ ), and adult patients with advanced disease categories (smoldering systemic mastocytosis [*SSM*],  $n = 5$ ; aggressive systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease [*ASM-AHNMD*],  $n = 3$ ) show increased tryptase levels compared with those with nonadvanced disease categories (CM/ISM,  $n = 28$ ; indolent systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease [*ISM-AHNMD*],  $n = 2$ ). Data are presented as box plots (minimum to maximum) with medians (*left*) and scatter dot plots with means (*right*). Statistical analysis was performed with the nonparametric Mann-Whitney *U* test. **B**, IL-31 levels (*y-axis*) positively correlate with tryptase levels (*x-axis*) in all patients with mastocytosis ( $n = 50$ ). Correlation analysis was performed by using Spearman rank order correlation (Spearman  $r = 0.2708$ ,  $P = .0572$ ).

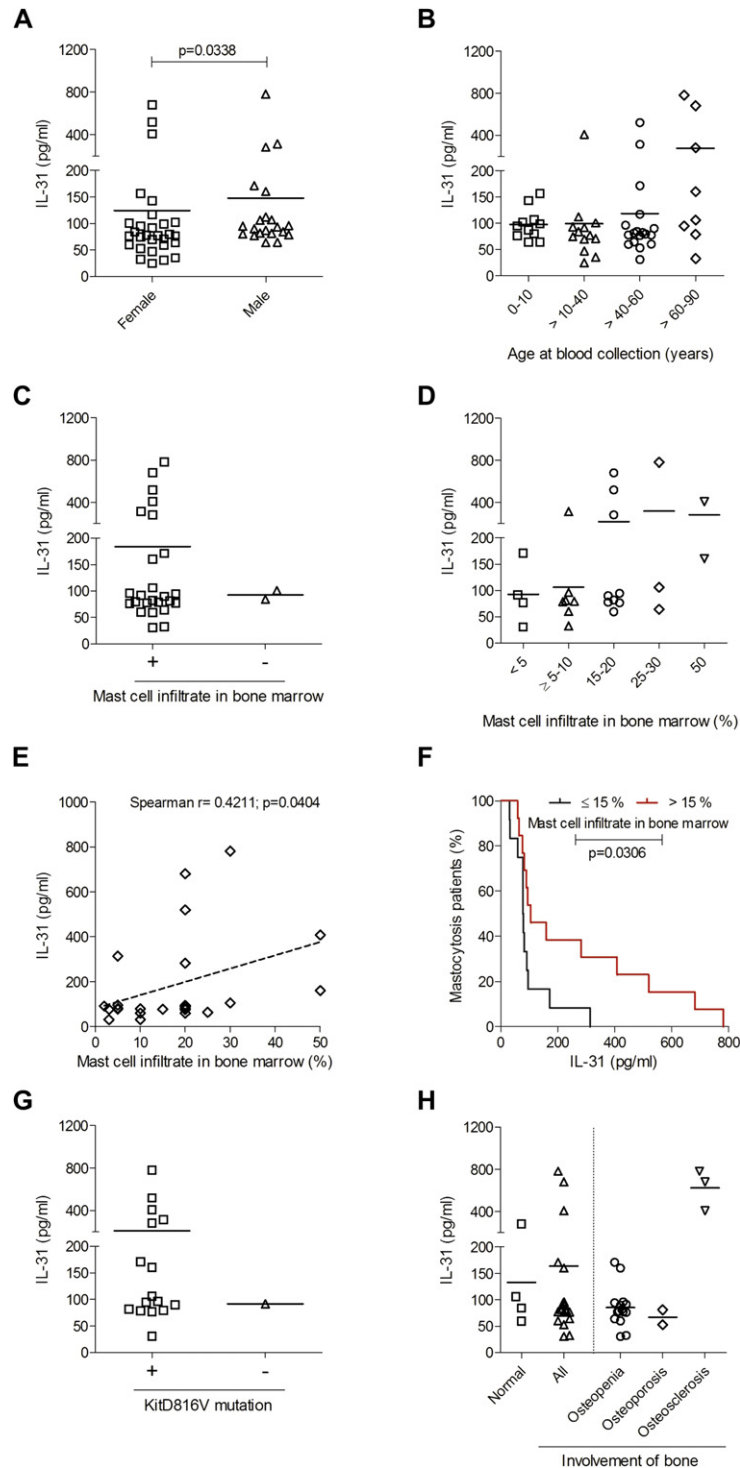


**FIG E2.** Serum IL-31 levels in patients with mastocytosis subdivided according to the following clinically relevant parameters: **A**, Sex ( $n = 50$ , Mann-Whitney  $U$  test). **B**, Age at blood collection ( $n = 50$ ). **C**, Presence of MC infiltrate in bone marrow ( $n = 27$ ). **D**, Percentage of MC infiltrate in bone marrow ( $n = 27$ ). **E**, IL-31 serum levels ( $y$ -axis) positively correlate with percentage of MC infiltrate in bone marrow ( $x$ -axis;  $n = 27$ ; Spearman rank order correlation). **F**, Patients with MC infiltrates of greater than 15% show significantly increased IL-31 levels compared with those with MC infiltrates of 15% or less ( $n = 27$ ; Kaplan-Meier curves using the log-rank test). **G**, Presence of KitD816V mutation ( $n = 17$ ). **H**, Presence of osteopenia, osteoporosis, or osteosclerosis ( $n = 24$ ). **I**, Duration from start of symptoms to blood collection ( $n = 50$ ). **J**, Duration from diagnosis to blood collection ( $n = 50$ ). **K**, Presence of pruritus ( $n = 50$ ). **L**, Presence of anaphylaxis ( $n = 50$ ). **M**, Presence of flush ( $n = 50$ ). **N**, Presence of diarrhea ( $n = 50$ ). **O**, Presence of skin lesions ( $n = 50$ ). **P**, Intake of  $H_1$  antihistamines at blood collection ( $n = 50$ ).

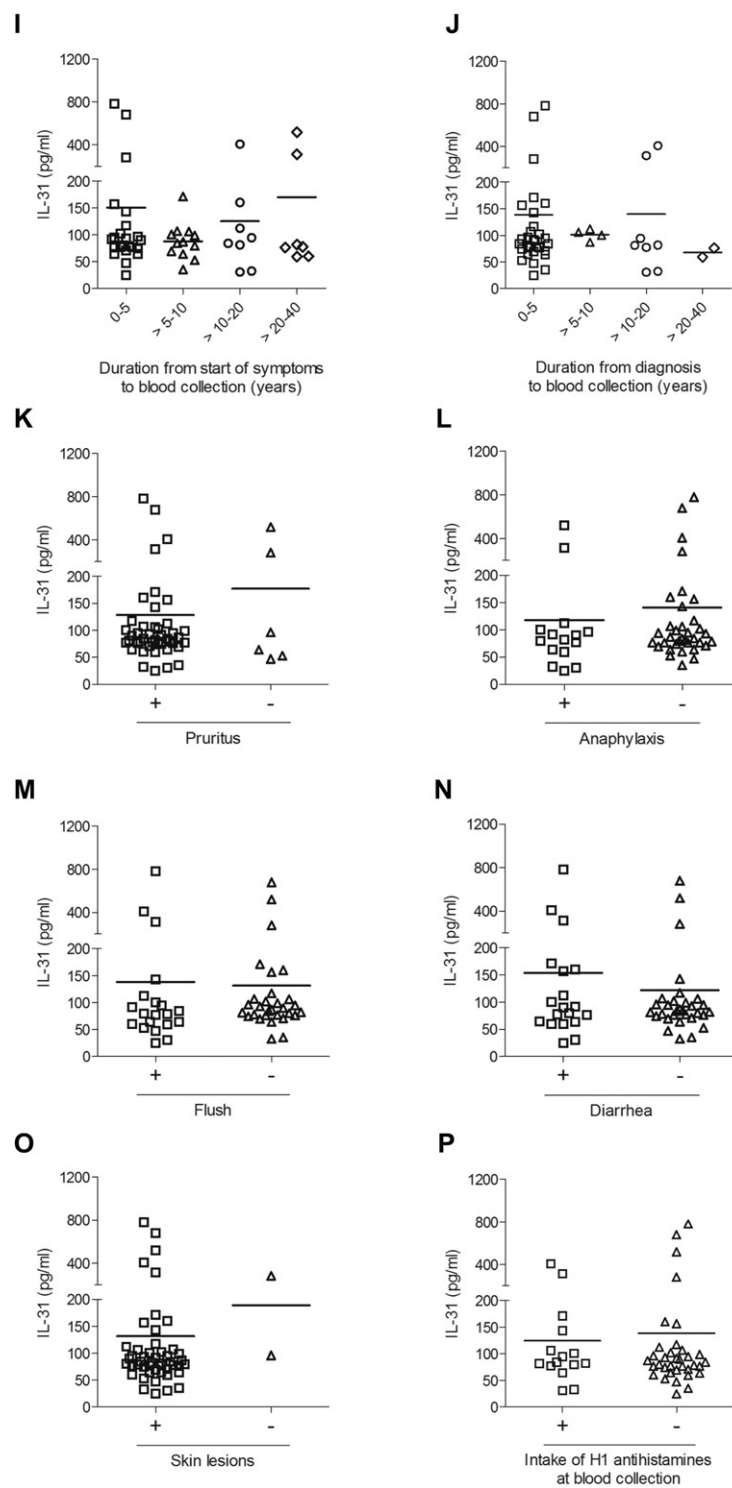


FIG E2. (Continued)

**TABLE E1.** Clinical characteristics of patients with mastocytosis participating in the study

| Parameters                                                       | Patients         |                    |                   |                              |                           |
|------------------------------------------------------------------|------------------|--------------------|-------------------|------------------------------|---------------------------|
|                                                                  | All, no. (%)     | Pediatric, no. (%) | Adult             |                              |                           |
|                                                                  |                  |                    | All, no. (%)      | Nonadvanced disease, no. (%) | Advanced disease, no. (%) |
| All                                                              | 50 (100%)        | 12 (24.0%)         | 38 (76.0%)        | 30 (78.9%)                   | 8 (21.1%)                 |
| Sex                                                              |                  |                    |                   |                              |                           |
| Male                                                             | 21 (42.0%)       | 7 (58.3%)          | 14 (36.8%)        | 10 (33.3%)                   | 4 (50%)                   |
| Female                                                           | 29 (58.0%)       | 5 (41.7%)          | 24 (63.2%)        | 20 (66.7%)                   | 4 (50%)                   |
| Age at collection of blood sample (y)                            |                  |                    |                   |                              |                           |
| Mean $\pm$ SD                                                    | 37.2 $\pm$ 23.1  | 5.4 $\pm$ 4.4      | 47.3 $\pm$ 16.5   | 43.1 $\pm$ 14.4              | 62.9 $\pm$ 14.8           |
| Range                                                            | 1-82             | 2-16               | 17-82             | 17-77                        | 37-82                     |
| Tryptase ( $\mu$ g/L)                                            |                  |                    |                   |                              |                           |
| Mean $\pm$ SD                                                    | 91.0 $\pm$ 120.6 | 18.2 $\pm$ 17.4    | 114.0 $\pm$ 130.1 | 54.6 $\pm$ 52.2              | 337.0 $\pm$ 78.6          |
| Range                                                            | 2.8-483.0        | 2.8-66.5           | 4.4-483.0         | 4.4-165.0                    | 249.0-483.0               |
| Category of mastocytosis                                         |                  |                    |                   |                              |                           |
| CM/ISM                                                           | 40 (80.0%)       | 12 (100%)          | 28 (73.7%)        | 28 (93.3%)                   | 0 (0.0%)                  |
| ISM-AHNMD                                                        | 2 (4.0%)         | 0 (0.0%)           | 2 (5.3%)          | 2 (6.7%)                     | 0 (0.0%)                  |
| SSM                                                              | 5 (10.0%)        | 0 (0.0%)           | 5 (13.1%)         | 0 (0.0%)                     | 5 (62.5%)                 |
| ASM-AHNMD                                                        | 3 (6.0%)         | 0 (0.0%)           | 3 (7.9%)          | 0 (0.0%)                     | 3 (37.5%)                 |
| Presence of MC infiltrate in bone marrow (n = 27)                | 25/27 (92.6%)    | 0/0 (0.0%)         | 25/27 (92.6%)     | 17/19 (89.5%)                | 8/8 (100%)                |
| Presence of KitD816V mutation (n = 17)                           | 16/17 (94.1%)    | 0/0 (0.0%)         | 16/17 (94.1%)     | 9/10 (90.0%)                 | 7/7 (100%)                |
| Presence of osteopenia, osteoporosis, or osteosclerosis (n = 24) | 20/24 (83.3%)    | 0/0 (0.0%)         | 20/24 (83.3%)     | 14/17 (82.4%)                | 6/7 (85.7%)               |
| Disease duration (y), from start of symptoms to blood collection |                  |                    |                   |                              |                           |
| Mean $\pm$ SD                                                    | 9.4 $\pm$ 8.5    | 4.9 $\pm$ 4.3      | 10.8 $\pm$ 9.1    | 9.9 $\pm$ 9.1                | 14.0 $\pm$ 9.0            |
| Range                                                            | 0-31             | 1-16               | 0-31              | 0-31                         | 2-27                      |
| Disease duration (y), from diagnosis to blood collection         |                  |                    |                   |                              |                           |
| Mean $\pm$ SD                                                    | 6.3 $\pm$ 7.3    | 4.6 $\pm$ 3.2      | 6.7 $\pm$ 8.0     | 6.3 $\pm$ 8.3                | 8.6 $\pm$ 6.4             |
| Range                                                            | 0-31             | 1-10               | 0-31              | 0-31                         | 2-18                      |
| Presence of pruritus                                             | 44 (88.0%)       | 12 (100%)          | 32 (84.2%)        | 25 (83.3%)                   | 7 (87.5%)                 |
| Presence of anaphylaxis                                          | 15 (30.0%)       | 1 (8.3%)           | 14 (36.8%)        | 12 (40.0%)                   | 2 (25.0%)                 |
| Presence of flush                                                | 20 (40.0%)       | 4 (33.3%)          | 16 (42.1%)        | 13 (43.3%)                   | 3 (37.5%)                 |
| Presence of diarrhea                                             | 19 (38.0%)       | 4 (33.3%)          | 15 (39.5%)        | 12 (40.0%)                   | 3 (37.5%)                 |
| Presence of skin lesions                                         | 48 (96.0%)       | 12 (100%)          | 36 (94.7%)        | 28 (93.3%)                   | 8 (100%)                  |
| Intake of H <sub>1</sub> antihistamines at blood collection      | 15 (30.0%)       | 2 (16.7%)          | 13 (36.1%)        | 9 (30.0%)                    | 4 (50.0%)                 |

ASM-AHNMD, Aggressive systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease; ISM-AHNMD, indolent systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease; SSM, smoldering systemic mastocytosis.