

CORRESPONDENCE

Contribution of highly sensitive diagnostic methods to the diagnosis of systemic mastocytosis in the absence of skin lesions

DOI:10.1111/j.1398-9995.2012.02850.x

We have read with interest the article entitled 'Tryptase and histamine metabolites as diagnostic indicators of indolent systemic mastocytosis without skin lesions' by Doormaal et al. (1). Results of this study related to the higher risk for systemic mastocytosis (SM) conferred by male gender, insect-induced anaphylaxis and tryptase levels support previous findings from our group, based on which we have proposed a score to predict for SM in patients with mast cell (MC) activation symptoms without skin lesions (2), such score being further validated (3) in the largest cohort of SM patients without urticaria pigmentosa (UP) ($n = 80$) published so far. On the basis of our findings in both studies, which Doormaal et al. failed to cite, we would like to comment on some of the specific methods used by the authors to rule out/diagnose SM that may impact their results and conclusions.

It is well established that indolent SM (ISM) patients without UP typically display a low MC burden, which translates into a substantial proportion of cases lacking bone marrow (BM) MC aggregates (2). Consequently, highly sensitive diagnostic methods including multiparameter flow cytometry immunophenotyping for the identification of BMMC down to frequencies as low as $<10^{-4}$ to 10^{-5} , and the *KIT* mutation analysis of highly purified BMMC (4), should be routinely applied in these patients to avoid false-negative results. In fact, 10% of our ISM patients without UP showed $<0.01\%$ [the detection limit reported by the authors (1)] immunophenotypically aberrant BMMC (3), and even higher frequencies (18%) have been reported by Bonadonna et al. (5) in a series of 17 ISM patients without UP presenting with insect venom anaphylaxis. In addition, 11/78 patients (14%) from our series showed *KIT*-mutated BMMC in the absence of criteria for SM, which currently leads to the diagnosis of the so-called clonal MC activation syndrome (c-MCAS) or monoclonal MC activation syndrome (MMAS) (2,6); noteworthy, more than half (6/11) of these patients showed $<0.01\%$ of BMMC by flow cytometry. Investigation of the *KIT* mutation in whole (unfractionated) BM samples, specifically for the identification of the D816V *KIT* mutation without investigating other exon 17 mutations, as conducted by Doormaal et al. (1) may lead to a lower rate of detection of mutated

BMMC as previously demonstrated (4). In fact, in their study, Doormaal et al. (1) reported an overall frequency of *KIT* mutation of 85% among the SM group, a frequency significantly lower than that found in our series where molecular studies were performed in highly purified BMMC: overall frequency of *KIT* mutation among evaluable cases of 98%, including 7% of cases with *KIT* mutations involving exon 17 other than the D816V *KIT* mutation (3), supporting the need to screen for mutations in the entire exon 17 of *KIT* in cases lacking the classical D816V *KIT* mutation.

Finally, we would also like to support the authors' conclusion that serum tryptase levels $<20 \mu\text{g/l}$ exclude neither SM nor MMAS and that such cut-off could be lowered (5). However, caution should be taken in systematically refraining from BM examination in suspected patients with serum tryptase $<10 \mu\text{g/l}$, as proposed by the authors. In fact, 4/14 (28%) patients who presented with MC mediator release symptoms without UP and tryptase $<10 \mu\text{g/l}$ referred to our centre were finally diagnosed with SM ($n = 2$) and MMAS ($n = 2$), when the above-described diagnostic methods were followed (3). Consequently, neither SM nor MMAS can be ruled out, as proposed by the authors, based exclusively on both normal histology and cytology, and we strongly recommend that such BM studies are performed in experienced reference centres where the most sensitive diagnostic procedures available nowadays are routinely applied.

Conflict of interest

None.

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REPLY

In response to their comments on our paper (1) we like to mention that, first of all, with our aim to study the role of tryptase and histamine metabolites as diagnostic tools for indolent systemic mastocytosis (ISM), the focus was not to diagnose the monoclonal mast cell activation syndrome. Secondly, since December 2007, we used a new sensitive method to detect the D816V *KIT* mutation with a detection level of 0.001% mast cells in bone marrow aspirate. So far we found no false negative result (0/53), indicating that ISM with another *KIT* mutation than the D816V mutation is rare in our population of ISM patients with and without urticaria pigmentosa (UP). In only one case with UP and two minor diagnostic criteria, but without a *KIT* mutation, it is possible that we missed the diagnosis of SM because only the D816V *KIT* mutation was determined. Furthermore, we used in our study the WHO-criteria for the diagnosis of ISM; patients with normal bone marrow histology and cytology and tryptase levels below 20 µg/l did not fulfill the WHO-criteria, even if an exon 17 *KIT* mutation and CD25 expression would have been present. Looking retrospectively in our study at the patients without SM but a tryptase level >20 µg/l, we found one case in which in the presence of normal bone marrow histology and cytology the diagnosis of SM, although very unlikely, could not be excluded with 100% certainty according to the WHO criteria. This case would fulfill the SM criteria if another *KIT* mutation than the D816V mutation (we performed the new detection method in this case) and CD25 expression (we found <0.01% mast cells in flow cytometry) were present.

Alvarez-Twose et al. argued to be cautious in refraining from bone marrow examination if serum tryptase is below

10 µg/l, and mentioned two ISM patients with lower tryptase levels. Although we did not find any tryptase value below this threshold in the screening phase before bone marrow examination in the 53 ISM patients in our study, a decline in disease activity in ISM during follow-up is a well known phenomenon (2). Therefore, we thank the authors for their information, and we recommend not applying the threshold tryptase level of 10 µg/l too strictly in case of longstanding suspected symptoms.

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