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Diagnosis and classification of mastocytosis in non-specialized *versus* reference centres: a Spanish Network on Mastocytosis (REMA) study on 122 patients

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Summary

The diagnosis of 'rare diseases', such as mastocytosis, remains a challenge. Despite this, the precise benefits of referral of mastocytosis patients to highly specialized reference centres are poorly defined and whether patients should be managed at non-specialized *versus* reference centres remains a matter of debate. To evaluate the quality and efficiency of diagnostic procedures performed at the reference centres for mastocytosis in Spain (REMA) *versus* other non-reference centres, we retrospectively analysed a series of 122 patients, for the overall degree of agreement obtained for the World Health Organization (WHO) diagnostic and classification criteria betwen the referring and REMA centres. Our results showed that not all WHO diagnostic criteria were frequently investigated at the referring centres. Among the five WHO diagnostic criteria, the highest degree of agreement was obtained for serum tryptase levels [median 90% (95% confidence interval 84–96%)]; in turn, the overall agreement was significantly lower for the major histopathological criterion [80% (72–89%)], and the other three minor criteria: cytomorphology [68% (56–80%)] immunophenotyping of BM mast cells [75% (62–87%)] and detection of the KIT mutation [34% (8–60%)]. Referral of patients with diagnostic suspicion of mastocytosis to a multidisciplinary reference centre improves diagnostic efficiency and quality.

Keywords: systemic mastocytosis, reference centre, rare disease, flow cytometry, *KIT* mutation.

Mastocytosis is a rare clonal disorder characterized by the expansion and accumulation of pathological mast cells (MC) in distinct organs and tissues, such as the skin, gastrointestinal tract and/or the bone marrow (BM) (Valent *et al*, 2007; Horny *et al*, 2008). A prevalence of mastocytosis of 1 per 10 000 inhabitants has been reported, but under-diagnosis is assumed (Brockow, 2014). Although symptoms observed at presentation are mainly caused by MC infiltration and accumulation in the involved tissues and/or by the release of MC-mediators triggered by different stimuli (Akin & Metcalfe, 2004; Alvarez-Twose *et al*, 2010), they are very heterogeneous due to the variety of tissues involved, the specific patterns of involvement observed, and the broad spectrum of triggers for MC activation.

In most adult cases and in a significant fraction of childhood mastocytosis, the disease involves multiple tissues with systemic dissemination. According to the World Health Organization (WHO) (Horny *et al*, 2008), diagnosis of systemic mastocytosis (SM) requires coexistence of one major criterion (presence of multifocal dense aggregates of ≥15 MC in BM and/or other extracutaneous tissues) plus one minor criterion or, in the absence of the major criterion, simultaneous detection of at least three out of four minor criteria- (i) presence of morphologically atypical MC in smears or biopsy sections of BM or other extracutaneous organs, (ii) aberrant expression of CD25 and/or CD2 by BM MC, (iii) presence of the KIT D816V mutation in BM, blood or other extracutaneous organs, and (iv) serum tryptase levels >20 μg/l in the

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absence of other disorders associated with increased serum tryptase (Sperr *et al*, 2001a; Klion *et al*, 2003; Valent *et al*, 2008). Other clinical and biological findings and imaging data are also informative for subclassification of the disease (Valent *et al*, 2007; Horny *et al*, 2008). Overall, seven categories of mastocytosis are defined by the WHO classification, namely: (i) cutaneous mastocytosis (CM), (ii) extracutaneous mastocytoma, (iii) indolent SM (ISM), (iv) aggressive SM (ASM), (v) SM associated with another clonal haematological non-MC lineage disease (SM-AHNMD), (vi) MC leukaemia (MCL), and (vii) MC sarcoma(Valent *et al*, 2007; Horny *et al*, 2008). In addition, the WHO also defines another (provisional) diagnostic subtype of ISM -i.e. smouldering SM (SSM)- and two new subvariants of SM and ISM have been more recently recognized, i.e. well-differentiated SM (WDSM)(Akin *et al*, 2004; Garcia-Montero *et al*, 2006; Alvarez-Twose *et al*, 2015) and ISM in the absence of skin lesions ($ISMs^{-}$), respectively (Alvarez-Twose *et al*, 2010).

Diagnosis of 'rare diseases' in routine clinical practice still remains a challenge. This is particularly true for rare diseases such as mastocytosis, which require a multidisciplinary diagnostic approach together with highly-specialized laboratory diagnostic tests (Valent *et al*, 2007, 2011). Following the European Union recommendation for rare diseases, the European Competence Network on Mastocytosis (ECNM), an european network of Reference Centres and Centres of Excellence, has been created, to increase patient access to comprehensive high quality and cost-effective diagnostics and treatment of mastocytosis (Ayme & Rodwell, 2014). Among the ECNM Reference Centres, the Spanish Network on Mastocytosis (REMA) has become one of the most active and successful. At present, the REMA integrates two major units: a clinical reference centre (Instituto de Estudios de Mastocitosis de Castilla-La Mancha in Toledo; CLMast) which belongs to the public healthcare system of Spain, and a reference diagnostics laboratory at the Cancer Research Centre of the University of Salamanca (CIC; Salamanca, Spain). Patients from all over Spain are referred to the REMA, which acts as the reference centre for the National Health Care system in Spain with a total of 1722 patients (1346 adults and 376 children) being currently followed. Although most patients are referred to the REMA with the suspicion of suffering mastocytosis based on clinical symptoms and/or the presence of increased serum tryptase levels, there are still many patients that are referred only after a BM study and diagnosis had been established (or attempted) at the referring centres, sometimes even only when disease progresses to more advanced forms.

Here we report on a retrospective analysis of 122 patients who have been referred to the REMA with diagnostic suspicion of mastocytosis after a BM study had been performed at the referring (external) centre. Our major aim was to compare the results of the diagnostic work-up of BM specimens at the referring (non-specialized) centres with the final diagnosis made by the REMA.

Patients and methods

Patients

A total of 122 patients (61 women; 61 men) were retrospectively studied. As BM studies are not routinely performed in children, only adult patients (median age, 49 years; range 19–80 years), were selected for this analysis. The median time between the BM study at the referring centre and the REMA centre was 11 months (range: 12 d–13 years).

Inclusion criteria were: (i) adult patients referred to the REMA between May 2007 and March 2014 with suspicion or diagnosis of mastocytosis, (ii) BM study performed both at the centre of origin and at the time of referral to the REMA, and (iii) data available for all studies performed prior to REMA referral. For this purpose, the results of BM examination were retrospectively collected from the referring centres, directly from the clinical records provided by the patients or their medical doctors at the centre of origin. All patients gave their written informed consent to participate in the study according to the Declaration of Helsinki and the study was approved by the Institutional Ethics Committee of the Complejo Hospitalario de Toledo (Toledo, Spain).

Diagnostic work-up for mastocytosis at the REMA

At referral to the REMA, all 122 patients had a complete physical examination, blood cell count and differential, routine serum biochemistry tests and abdominal ultrasonography and/or computerized tomography (CT) scan. Osteoporosis was defined following well-established criteria (Miller, 2006) and presence of bone sclerosis -as assessed by skeletal X-ray survey and/or CT scan- was also recorded. Skin biopsy was performed in all cases with cutaneous lesions. Serum baseline tryptase (sBT; Phadia, Uppsala, Sweden) was measured in all patients at the time of BM biopsy.

In order to confirm mastocytosis, every patient underwent a new BM aspiration and biopsy at the REMA, following routine procedures that included systematic assessment of all WHO diagnostic criteria. Therefore, BM evaluation was performed following previously established criteria for morphology (Sperr *et al*, 2001b), histopathology, immunohistochemistry (Horny & Valent, 2001; Li, 2001), flow cytometry immunophenotyping (Teodosio *et al*, 2010), detection of *KIT* mutations (Sotlar *et al*, 2003; Garcia-Montero *et al*, 2006) and BM MC clonality, as previously reported in detail. Briefly, for morphological evaluation BM smears were stained with Wright-Giemsa and toluidine blue, and analysed by two independent haematopathologists using light microscopy. In each case, 25–100 MC were analysed and classified as described elsewhere (Sperr *et al*, 2001b). In addition, the presence of MC aggregates in BM particles was assessed in toluidine blue-stained samples; presence of focal or diffuse eosinophilia, as well as dysplastic features were also examined and recorded. In turn, BM biopsy sections were stained for

haematoxylin-eosin, giemsa, tryptase and KIT (c-kit), and analysed by three independent haematopathologists for overall cellularity, MC number and morphology, presence of compact MC aggregates, grade and type of MC infiltration, presence of fibrosis, bone sclerosis, and lymphoid aggregates. Immunophenotypical analysis of BM MC was routinely performed in parallel in the two REMA centres following REMA guidelines (Escribano *et al*, 2004) and a standard 6–8 colour flow cytometry antibody panel.(Morgado *et al*, 2014; Sanchez-Muñoz *et al*, 2014; Teodosio *et al*, 2015).

The *KIT* D816V mutation, as well as other *KIT* mutations localized at exon 17 (codons 814–819), were investigated on highly purified (≥97% purity) BM MC and other haematopoietic cell populations, as previously described (Sotlar *et al*, 2003; Garcia-Montero *et al*, 2006). In addition, identification of *KIT* mutations at exons 2, 9, 10, 11, 13, 14 and 15 was performed on genomic DNA by direct sequencing of amplified PCR products, using the dye-deoxy terminator method, and an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

Statistical methods

The SPSS 15.0 software (Chicago, IL, USA) was used for all statistical analyses. Median values, mean and standard deviation (SD), as well as range, were calculated for all continuous variables; for categorical variables, frequencies were used. Comparisons between groups were performed with either the non-parametric Kruskal–Wallis and Mann–Whitney *U* tests (for continuous variables) or the Pearson chi-square and Fisher exact tests (for categorical variables). The grade of correlation between two continuous variables was assessed by the coefficient of determination (R^2). Receiver operating curve (ROC) analysis was used to assess the sensitivity and specificity of each variable or combination of variables, for the diagnosis and classification of individual cases. The overall degree of agreement (OA) was calculated as the proportion of concordant cases from all cases analysed, and the statistical significance of such agreement was assessed by the McNemar chi-square test for paired categorical variables and the kappa coefficient (k) (proportion of agreement higher than that corresponding to a random agreement); based on the k-value, the degree of agreement of the results was classified as follows: (i) very low agreement: $k < 0{\cdot}20$, (ii) low agreement: $k \geq 0{\cdot}21$ and $\leq 0{\cdot}40$, (iii) moderate agreement: $k \geq 0{\cdot}41$ and $\leq 0{\cdot}60$, (iv) good: $k \geq 0{\cdot}61$ and $\leq 0{\cdot}80$, and (v) excellent agreement: $k \geq 0{\cdot}80$ and $\leq 1{\cdot}0$ (Fleiss, 1981); *P* values $<0{\cdot}05$ were considered to be associated with statistical significance.

Results

Number of diagnostic tests fulfilled and evaluable test results

Based on the diagnostic work-up performed at the referring centres, only 16/122 (13%) patients, had data available for all five WHO diagnostic criteria for SM (Table I and Fig 1); in 44 cases (36%) 4/5 criteria were studied, while ≤3 criteria were assessed in the remaining 62/122 (51%) patients. sBT was evaluated in 108 (89%) cases, the major histopathological criterion was assessed in 106 (87%) patients and MC cytomorphology in 86 (71%) cases, whereas the immunophenotype of BM MC and the presence of *KIT* mutation were investigated in whole BM sample in only 64 (53%) and 38 (31%) patients, respectively (Table I and Fig 1). In contrast, all WHO criteria were successfully assessed at the REMA in virtually every case (119/122 patients, 98%; $P < 0{\cdot}001$) (Table I) and 4/5 criteria were studied in the remaining three cases in whom *KIT* mutational analysis could not be performed because not enough BM MC were available. Thus, except for the *KIT* mutation, all the other four diagnostic criteria (sBT, presence of BM compact MC aggregates in the BM and an altered BM MC immunophenotype and morphology) were systematically investigated ($P < 0{\cdot}001$). In those 119/122 cases (98%), in whom the *KIT* mutation was investigated, both purified BM MC and other purified BM cell populations were independently assessed.

Discrepancies obtained for the diagnostic tests at the referring centres versus *the REMA*

Skin mastocytosis. Presence *versus* absence of cutaneous lesions typical of mastocytosis in the skin (MIS) was found to be concordant among the referring and REMA centres in 97% of cases, with only four discrepant cases ($P = 0{\cdot}63$). Of the latter four cases, one patient was found to have skin lesions at the REMA, but not diagnosed with MIS by the referring centre, probably because the lesions were very faint and a skin biopsy was not initially performed in this patient. In the other three patients, MIS was diagnosed at the referring centre, but no typical MIS lesions were observed in their skin, when the patients were referred to the REMA. As MIS could have disappeared from initial diagnosis before the patients were referred and studied at the REMA, skin biopsy specimens available at the referring centre were re-assessed; no histopathological evidence of MIS was found in any of these three cases.

Serum tryptase levels. The median sBT level at the referring centres was 38·4 ng/ml (range: 2·6–398 ng/ml); overall, such levels were very similar ($P = 0{\cdot}62$) to those obtained at referral to the REMA (median of 39·3 ng/ml; range: 2·3–598 ng/ml) (Figure S1), with a percentage agreement between the two groups of centres of 90% for the diagnostic sBT cut-off level of 20 ng/ml ($P = 1{\cdot}0$; $k = 0{\cdot}74$), despite the time elapsed between the studies (Figure S1). In 6/11 discrepant cases, sBT level <20 ng/ml was detected at first evaluation at the referring centre, while sBT >20 ng/ml was found at referral to the REMA with a median time lapse between studies of 23 months (range: 4–48 months). For the other five discordant cases, increased sBT (>20 ng/ml) levels were initially

Table I. Frequency of the WHO diagnostic criteria for SM obtained via examination of the BM in the patients studied (n = 122) at the referring *versus* the REMA centres.

	WHO major criterion	WHO minor criteria				Number of criteria evaluated		
	Immunohistochemical	Morphological	Immunophenotypical	*KIT* D816V mutation	Serum tryptase >20 ng/ml	5/5 criteria studied	4/5 criteria studied	≤3/5 criteria studied
Referring centre								
Total cases investigated	106 (87%)	86 (70%)	64 (52%)	38 (31%)*	108 (89%)	16 (13%)	44 (36%)	62 (51%)
Non-evaluable	2 (1·8%)	3 (3·4%)	4 (6·2%)	0	0			
Positive	68 (65%)	49 (59%)	40 (67%)	11 (29%)	78 (72%)			
Negative	36 (35%)	34 (41%)	20 (33%)	27 (71%)	30 (28%)			
REMA centre								
Total cases investigated	122 (100%)	122 (100%)	122 (100%)	119 (97·5%)$^{+}$	122 (100%)	119 (98%)	3 (2%)	0
Non-evaluable	3 (2·5%)	4 (3·3%)	1 (0·8%)	3 (2·5%)	0			
Positive	89 (73%)	102 (84%)	105 (86%)	104 (87%)	89 (73%)			
Negative	30 (25%)	16 (13%)	16 (13%)	15 (13%)	33 (27%)			
p-value ~ (investigated *versus* non investigated cases)	**0·00002**	**<0·00001**	**<0·00001**	**<0·00001**	**0·00008**	**<0·00001**	**<0·00001**	**<0·00001**
p-value ~ (positive *versus* negative cases)	**0·15**	**0·00002**	**0·0002**	**<0·00001**	**0·90**			

BM, bone marrow; *P*, p-value; REMA: Spanish network on mastocytosis; SM, systemic mastocytosis; WHO, World Health Organization.

*Analysed in whole BM samples; +systematically analysed in fluorescence-activated cell sorted-purified BM mast cells and other purified BM cell populations. ~*P* values <0·05 were considered to be associated with statistical significance.

found at the referring centre, but they were not confirmed during the REMA diagnostic work-up. The latter discrepancies (particularly marked in 2/5 cases) could be due to the fact that either sBT levels had not been initially analysed during a basal situation of the disease, but during an exacerbation of the MC-mediator release symptoms or because patients had been treated with agents that block the release of MC mediators.

BM histopathology. Full concordance between the results of the evaluation of the WHO major criterion at the referring and the REMA centres was observed in 80% of cases (P = 0·01; k = 0·51) (Table II). In 16/20 discrepant patients, no BM MC aggregates were found at the referring centre, while their presence was confirmed at the REMA; the opposite was found in the remaining 4 patients. Re-evaluation of BM specimens at the referring centre was performed in 3/16 discrepant cases from the former group of cases and revealed presence of BM MC aggregates as per the WHO criterion in all three patients.

BM MC cytomorphology. Although cytomorphology is routinely available at most diagnostic haematology laboratories and it is not a complex technique, cytomorphological assessment of BM MC was only performed in 70% of cases at the referring centres (P < 0·001). Among those cases assessed for MC cytomorphology at both sites, the degree of agreement obtained between the referring and the REMA centres as regards the presence of an altered BM MC morphology according to the WHO criterion, was relatively low (68%; P < 0·001; k = 0·23) (Table II). In two discrepant cases, BM MC morphology was evaluated as compatible with mastocytosis at the referring centres, while no abnormal MC were detected in the BM specimen at the REMA; also, one of these two cases was diagnosed as having SM based only on the morphological criterion and a sBT level <20 ng/ml; this diagnosis was ruled out at the REMA based on the absence of any WHO criteria compatible with mastocytosis. In turn, in another 24 cases where an abnormal MC morphology was found at the REMA, this criterion was not compatible with mastocytosis at the referring centre.

BM MC immunophenotype. An overall concordance of 75% was found between the two (referring and REMA) groups of centres (P = 0·0074; P = 0·31) (Table II). However, in almost half of the cases (58/122; 48%) this criterion was not assessed at the referring centres (P < 0·001). Regarding discrepant cases, two patients had an immunophenotype compatible with mastocytosis at the referring centre, which was not confirmed at the REMA. Despite in one of these patients, the immunophenotype of BM MC was the only WHO compatible criteria, the final diagnosis was SM; when this patient was referred to the REMA, all WHO criteria were investigated but none showed a compatible result, thereby, the diagnosis of SM was ruled out. The second patient was a case

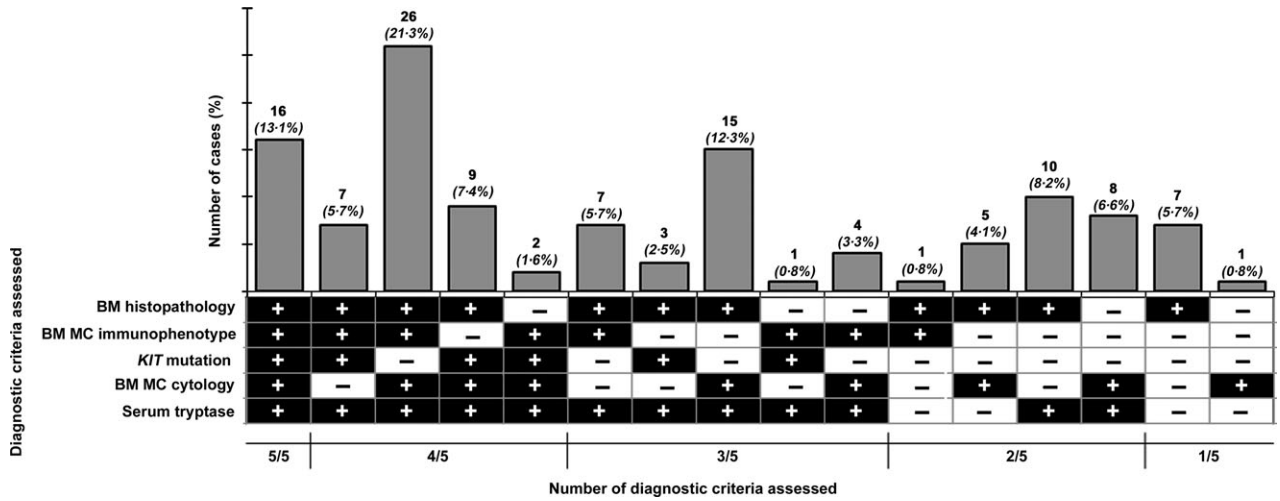

Fig 1. Patient distribution according to the combination of the diagnostic criteria evaluated at the referring centres. BM, bone marrow; MC, mast cell.

Table II. Percentage agreement for the different WHO BM diagnostic criteria for systemic mastocytosis obtained at the referring *versus* the REMA centres.

Criterion	Overall agreement % (95% CI)	P-value (McNemar chi-square test)	Kappa coefficient	Overall agreement score
Immunohistochemical	80% (72–89%)	0·01	0·51	moderate
Morphological	68% (56–80%)	<0·0001	0·23	low
Immunophenotypical	75% (62–87%)	0·007	0·31	low
KIT D816V+ mutation	34% (8–60%)	<0·0001	0·04	very low
Serum tryptase >20 ng/ml	90% (84–96%)	1·00	0·74	good

The overall degree of agreement (OA) was calculated as the proportion of true positive plus true negative cases from all cases analyzed; the statistical significance of such agreement was assessed by the kappa coefficient (k) (proportion of agreement higher than that corresponding to a random agreement) and the results were classified as follows: (i) very low agreement: $k < 0{\cdot}20$, (ii) low agreement: $k \geq 0{\cdot}21$ and $\leq 0{\cdot}40$, (iii) moderate agreement, $k \geq 0{\cdot}41$ and $\leq 0{\cdot}60$, (iv) good: $k \geq 0{\cdot}61$ and $\leq 0{\cdot}80$, and (v) excellent agreement: $k \geq 0{\cdot}80$ and $\leq 1{\cdot}0$ (Fleiss, 1981).
BM, bone marrow; CI, confidence interval; REMA, Spanish Network on Mastocytosis; WHO, World Health Organization.

of MCL, a condition where the BM MC immunophenotype can be normal (CD2− and CD25−/dimly+). In turn, there were 13 cases with an immunophenotype compatible with mastocytosis at the REMA, that were not described as such at the referring centres; all these cases also had the *KIT* mutation, their final diagnosis being ISM in 12 patients (one of them without skin lesions) and ASM in the remaining case, with a median percentage of BM MC infiltration by flow cytometry of 0·14%; the specific diagnoses of these 13 cases at the referring centres were: CM ($n = 4$), ISM ($n = 8$) and ASM ($n = 1$).

BM MC KIT mutational status. At the referring centres, *KIT* mutation was only assessed in BM samples from 38 cases (31%; $P < 0{\cdot}001$); in all these cases whole BM specimens, but not purified BM MC populations were evaluated, which frequently leads to a lower sensitivity of detection.(Garcia-Montero *et al*, 2006) In fact, the *KIT* mutation was found in only 11/38 cases analysed at the referring centres, while 36/38 of these cases showed *KIT* mutation when analysed at the REMA centres (29% vs. 95%; $P < 0{\cdot}001$) (Table III).

Differences in patient treatment

In order to determine the impact of the above-described discrepancies obtained during the diagnostic work-up for mastocytosis on the clinical management of the patients, we further investigated differences in the therapies administered for the same patient cohort. For this purpose, patients were subdivided into five different treatment groups: (i) those who received no therapy, (ii) those treated with oral sodium cromolyn alone, (iii) patients who received histamine receptor blockers, (iv) cases treated with intensive anti-mediator therapy (sodium cromolyn plus histamine receptor blockers, corticosteroids or leukotriene (LT) antagonists), and (v) those who underwent therapy with cytoreductive and/or

Table III. Degree of agreement regarding the *KIT* mutational status of SM patients obtained at the referring *versus* the REMA centres.

KIT mutational status				
	REMA centres†			
Referring centre*	Negative	MC-restricted mutation	Multilineal myeloid mutation	Multilineal myeloid and lymphoid mutation
Negative	2 (100%)	20 (91%)	2 (25%)	3 (100%)
Positive	0 (0%)	2 (9%)	6 (75%)	3 (100%)

BM, bone marrow; MC, mast cell; REMA, Spanish Network on Mastocytosis; SM, systemic mastocytosis.

*Assessed in whole BM samples.

†Analysed in fluorescence-activated cell sorted-purified BM MC and other BM cell populations.

immunomodulatory drugs. At the referring centres, 46 cases (38%) did not receive any treatment *versus* only 6 patients (5%) at the REMA ($P < 0{\cdot}001$). Consequently, the number of patients receiving oral sodium cromolyn alone or in combination with antihistamines was 97 (80%) at the REMA *versus* only 23 (19%) at the referring centres ($P < 0{\cdot}001$). In contrast, patients treated with only histamine receptor blockers comprised the most numerous group at the referring centres, (45 cases; 37%) *versus* only 6 (5%) cases at the REMA ($P < 0{\cdot}001$). No significant differences ($P = 0{\cdot}25$) were found as regards the other two treatment groups (Table SI).

Discussion

The diagnosis of mastocytosis requires a multidisciplinary work-up with the involvement of different groups of medical experts. This is due to the fact that, despite that the majority of patients present with dermatological symptoms, in a substantial fraction of the cases the disease emerges as a MC activation syndrome or a malignant haematological disorder, frequently in the absence of skin lesions (Bonadonna *et al*, 2009; Alvarez-Twose *et al*, 2010, 2012; Valent *et al*, 2011; Matito *et al*, 2014). Moreover, the last decade has seen a great improvement in the diagnosis of SM due to the development and use of an increasingly broad panel of more sensitive and specific techniques to assess BM involvement [e.g. by flow cytometry immunophenotyping and polymerase chain reaction (PCR)-based molecular assays] (Valent *et al*, 2007; Horny *et al*, 2008; Teodosio *et al*, 2010; Sanchez-Munoz *et al*, 2011a), in addition to the more conventional assays. Due to the lack of solid evidence about the quality and efficiency of the diagnostic work-up, treatment and outcome achieved in both settings, the need for reference centres that are highly experienced in mastocytosis still remains a matter of debate in developed countries.

Here we evaluated the quality and efficiency of the diagnostic procedures performed at the reference centres for mastocytosis in Spain (REMA) *versus* referring centres, which do not have a dedicated clinical mastocytosis unit. To the best of our knowledge, this is the first study in which the agreement obtained for the WHO diagnostic and classification criteria, as well as the treatment administered, have been compared between the two groups of centres for a large cohort of mastocytosis patients.

Overall, our results showed a significantly better performance at the reference *versus* the referring centres. Thus, at the centres not specialized in mastocytosis, not all WHO diagnostic criteria were usually investigated, which may potentially reflect the lack of awareness about the disease. In addition, the lack of diagnostic criteria at the referring centres was also frequently associated with inappropriate diagnostic assay performance. For example, BM biopsy was frequently performed without a BM aspiration being performed in parallel, making the assessment of the minor diagnostic BM criteria more difficult; in addition, immunophenotypic characterization of BM MC was frequently restricted to markers that are useful to identify BM MC (e.g. CD117), but it did not include evaluation of truly diagnostic markers, such as CD25 and/or CD2; finally, the *KIT* mutation was only investigated at the referring centres in around one-third of cases. Consequently, only one-third of the cases matched the correct WHO diagnostic subtype of mastocytosis at the referring centres.

Despite the limited diagnostic efficiency observed at the referring centres, the results obtained also reveal a lack of awareness about the disease and its behaviour. In this regard, accumulated evidence showed that patients with MIS and an adult onset have a very high probability (>95%) for systemic disease (Berezowska *et al*, 2014); thus, MIS is of high predictive value for SM with BM involvement in adults. However, due to the lower MC tumour load in the BM of these patients, usage of high-sensitive flow cytometry immunophenotyping and molecular assays is critical for the efficient diagnosis of SM (Sanchez-Muñoz *et al*, 2011b; Alvarez-Twose *et al*, 2012). In fact, in cases presenting with low BM MC numbers (e.g. <0·01%), *KIT* mutational analysis should be performed on highly-purified MC(Garcia-Montero *et al*, 2006; Kristensen *et al*, 2014) and/or by sensitive allele specific-oligonucleotide PCR (ASOqPCR) techniques, in order to overcome the limited sensitivity associated with the conventional molecular assays performed at the referring centres for other purposes.

In the last decade it has been confirmed that the presence of multilineage involvement of haematopoiesis by the *KIT*

mutation is the most relevant independent prognostic factor for progression of ISM into a more advanced disease subtype (e.g. ASM, acute myeloid leukaemia and/or myelodysplastic syndrome).(Escribano *et al*, 2009; Teodosio *et al*, 2013) Despite this, multilineage involvement of haematopoiesis by the *KIT* mutation was never investigated in our cohort at the referring centres; on the contrary, it was systematically evaluated in unfractionated whole BM samples and not in purified BM cell populations. Consequently, this lead to inappropriate and, at least, incomplete prognostic assessment of individual ISM patients at diagnosis (Garcia-Montero *et al*, 2006; Escribano *et al*, 2009). This may be due to lack of the appropriate (specialized) diagnostic approaches required for assessment of multilineage BM involvement by the *KIT* mutation at non-specialized centres in Spain. In contrast, the relatively high frequency of misdiagnosed cases and undertreated patients with, e.g. limited usage of oral sodium cromolyn, can only be due to a potential lack of local experience in the management of mastocytosis.

Therefore, we encourage the medical community not to perform invasive techniques if the centre is not specialized in the diagnosis of this disease, particularly if the centre does not have access to all up-to-date diagnostic tests. Otherwise, this will only increase the diagnostic costs and delay diagnosis with potential consequences regarding patient quality of life.

In summary, here we show for the first time that referral of patients with a diagnostic suspicion of mastocytosis to a multidisciplinary reference centre improves diagnostic efficiency and quality. At the same time it may avoid repeated studies, with ultimately decreased costs. Overall, these results point out the need for highly-specialized reference centres for the diagnosis and management of mastocytosis patients, in line with the recommendations of the European Council for rare diseases (Ayme & Rodwell, 2014).

Author contributions

L.S-M. performed the research, analysed the data, interpreted results and wrote the paper; JM.T.M. analysed the data, interpreted results, made the figures and critically reviewed the paper; I. A-T. and A.M. performed the clinical follow-up of the patients and critically reviewed the paper; AC. G-M. and C.T. analysed the data, interpreted results and critically reviewed the paper; M. J-A. performed *KIT* mutation experiments and critically reviewed the paper; A. M. and C.C. contributed with technical support and critically reviewed the paper; D. G-O. performed the clinical follow-up of the patients and critically reviewed the paper; M. M. performed histopathological studies and critically reviewed the paper; L.E. and A.O. supervised the study, interpreted results and critically reviewed the paper.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Correlation between serum tryptase levels evaluated at the referring center and at the REMA (panel A), and between the percentage in serum tryptase differences observed for the two assessments and the time lapse (days) between them (panel B).

Table SI. Distribution of the patients studied (n = 122) according to the type of therapy received in the referring and the REMA center.

References

Akin, C. & Metcalfe, D.D. (2004) Systemic mastocytosis. *Annual Review of Medicine*, **55**, 419–432.

Akin, C., Fumo, G., Yavuz, A.S., Lipsky, P.E., Neckers, L. & Metcalfe, D.D. (2004) A novel form of mastocytosis associated with a transmembrane c-kit mutation and response to imatinib. *Blood*, **103**, 3222–3225.

Alvarez-Twose, I., Gonzalez de Olano, D., Sanchez-Munoz, L., Matito, A., Esteban-Lopez, M.I., Vega, A., Mateo, M.B., Diaz, Alonso, de Durana, M.D., de la Hoz, B., Del Pozo, M.D., Caballero, T., Rosado, A., Sanchez-Matas, I., Teodosio, C., Jara-Acevedo, M., Mollejo, M., Garcia-Montero, A., Orfao, A. & Escribano, L. (2010) Clinical, biological and molecular characteristics of systemic mast cell disorders presenting with severe mediator-related symptoms. *The Journal of Allergy and Clinical Immunology*, **125**, 1269–1278.

Alvarez-Twose, I., Matito, A., Sanchez-Munoz, L., Morgado, J.M., Orfao, A., Escribano, L., Van Doormaal, J.J., van der Veer, E., van Voorst Vader, P.C., Kluin, P.M., Mulder, A.B., van der Heide, S., Arends, S., Kluin-Nelemans, J.C., Oude Elberink, J.N. & de Monchy, J.G. (2012) Contribution of highly sensitive diagnostic methods to the diagnosis of systemic mastocytosis in the absence of skin lesions. *Allergy*, **67**, 1190–1191.

Alvarez-Twose, I., Jara-Acevedo, M., Morgado, J.M., Garcia-Montero, A., Sanchez-Munoz, L., Teodosio, C., Matito, A., Mayado, A., Caldas, C., Mollejo, M., Orfao, A. & Escribano, L. (2015) Clinical, immunophenotypic, and molecular characteristics of well-differentiated systemic mastocytosis. *The Journal of Allergy and Clinical Immunology*, **2015**, doi: 10.1016/j.jaci.2015.05.008

Ayme, S. & Rodwell, C. (2014) The European Union Committee of Experts on Rare Diseases: three productive years at the service of the rare disease community. *Orphanet Journal of Rare Diseases*, **9**, 30.

Berezowska, S., Flaig, M.J., Rueff, F., Walz, C., Haferlach, T., Krokowski, M., Kerler, R., Petat-Dutter, K., Horny, H.P. & Sotlar, K. (2014) Adult-onset mastocytosis in the skin is highly suggestive of systemic mastocytosis. *Modern Pathology*, **27**, 19–29.

Bonadonna, P., Perbellini, O., Passalacqua, G., Caruso, B., Colarossi, S., Dal, F.D., Castellani, L., Bonetto, C., Frattini, F., Dama, A., Martinelli, G., Chilosi, M., Senna, G., Pizzolo, G. & Zanotti, R. (2009) Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. *The Journal of Allergy and Clinical Immunology*, **123**, 680–686.

Brockow, K. (2014) Epidemiology, prognosis, and risk factors in mastocytosis. *Immunology and Allergy Clinics of North America*, **34**, 283–295.

Escribano, L., Diaz-Agustin, B., López, A., López, R.N., García-Montero, A., Almeida, J., Prados, A., Angulo, M., Herrero, S., Orfao, A. & Spanish, N.M. (2004) Immunophenotypic analysis of mast cells in mastocytosis: when and how to do it. Proposals of the Spanish network on mastocytosis (REMA). *Cytometry B Clinical Cytometry*, **58B**, 1–8.

Escribano, L., Alvarez-Twose, I., Sanchez-Munoz, L., Garcia-Montero, A., Nunez, R., Almeida, J., Jara-Acevedo, M., Teodosio, C., Garcia-Cosio, M., Bellas, C. & Orfao, A. (2009) Prognosis in adult indolent systemic mastocytosis: a long-term study of the Spanish Network on Mastocytosis in a series of 145 patients. *The Journal of Allergy and Clinical Immunology*, **124**, 514–521.

Fleiss, J. (1981) The measurements of interrater agreement. In: Statistical Methods for Rates and Proportions, 2nd edn. pp. 212–304. John Wiley & Sons, New York.

Garcia-Montero, A.C., Jara-Acevedo, M., Teodosio, C., Sanchez, M.L., Nunez, R., Prados, A., Aldanondo, I., Sanchez, L., Dominguez, M., Botana, L.M., Sanchez-Jimenez, F., Sotlar, K., Almeida, J., Escribano, L. & Orfao, A. (2006) KIT mutation in mast cells and other bone marrow haematopoietic cell lineages in systemic mast cell disorders. A prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Blood*, **108**, 2366–2372.

Horny, H.P. & Valent, P. (2001) Diagnosis of mastocytosis: general histopathological aspects, morphological criteria, and immunohistochemical findings. *Leukemia Research*, **25**, 543–551.

Horny, H.P., Metcalfe, D.D., Bennet, J.M., Bain, B.J., Akin, C., Escribano, L. & Valent, P. (2008) Mastocytosis. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds. by S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 54–63. IARC, Lyon.

Klion, A.D., Noel, P., Akin, C., Law, M.A., Gilliland, D.G., Cools, J., Metcalfe, D.D. & Nutman, T.B. (2003) Elevated serum tryptase levels identify a subset of patients with a myeloproliferative variant of idiopathic hypereosinophilic syndrome associated with tissue fibrosis, poor prognosis, and imatinib responsiveness. *Blood*, **101**, 4660–4666.

Kristensen, T., Vestergaard, H., Bindslev-Jensen, C., Moller, M.B. & Broesby-Olsen, S. (2014) Sensitive KIT D816V mutation analysis of blood as a diagnostic test in mastocytosis. *American Journal of Hematology*, **89**, 493–498.

Li, C.Y. (2001) Diagnosis of mastocytosis: value of cytochemistry and immunohistochemistry. *Leukemia Research*, **25**, 537–541.

Matito, A., Alvarez-Twose, I., Morgado, J.M., Sanchez-Munoz, L., Orfao, A. & Escribano, L. (2014) Anaphylaxis as a clinical manifestation of clonal mast cell disorders. *Current Allergy and Asthma Reports*, **14**, 450.

Miller, P.D. (2006) Guidelines for the diagnosis of osteoporosis: T-scores vs fractures. *Reviews in Endocrine and Metabolical Disorders*, **7**, 75–89.

Morgado, J.M., Sanchez-Munoz, L., Teodosio, C. & Escribano, L. (2014) Identification and immunophenotypic characterization of normal and pathological mast cells. *Methods in Molecular Biology*, **1192**, 205–226.

Sanchez-Munoz, L., Alvarez-Twose, I., Garcia-Montero, A.C., Teodosio, C., Jara-Acevedo, M., Pedreira, C.E., Matito, A., Morgado, J.M., Sanchez, M.L., Mollejo, M., Gonzalez-de-Olano, D., Orfao, A. & Escribano, L. (2011a) Evaluation of the WHO criteria for the classification of patients with mastocytosis. *Modern Pathology*, **24**, 1157–1168.

Sanchez-Munoz, L., Teodosio, C., Morgado, J.M. & Escribano, L. (2011b) Immunophenotypic characterization of bone marrow mast cells in mastocytosis and other mast cell disorders. *Methods in Cell Biology*, **103**, 333–359.

Sanchez-Munoz, L., Teodosio, C., Morgado, J.M., Perbellini, O., Mayado, A., varez-Twose, I., Matito, A., Jara-Acevedo, M., Garcia-Montero, A.C., Orfao, A. & Escribano, L. (2014) Flow cytometry in mastocytosis: utility as a diagnostic and prognostic tool. *Immunology and Allergy Clinics of North America*, **34**, 297–313.

Sotlar, K., Escribano, L., Landt, O., Möhrle, S., Herrero, S., Torrelo, A., Lass, U., Horny, H.P. & Bültmann, B. (2003) One-step detection of c-kit point mutations using PNA-mediated PCR-clamping and hybridization probes. *American Journal of Pathology*, **162**, 737–746.

Sperr, W.R., Jordan, J.H., Baghestanian, M., Kiener, H.P., Samorapoompichit, P., Semper, H., Hauswirth, A., Schernthaner, G.H., Chott, A., Natter, S., Kraft, D., Valenta, R., Schwartz, L.B., Geissler, K., Lechner, K. & Valent, P. (2001a) Expression of mast cell tryptase by myeloblasts in a group of patients with acute myeloid leukemia. *Blood*, **98**, 2200–2209.

Sperr, W., Escribano, L., Jordan, J.H., Schernthaner, G.H., Kundi, M., Horny, H.P. & Valent, P. (2001b) Morphologic properties of neoplastic mast cells: delineation of stages of maturation and implication for cytological grading of mastocytosis. *Leukemia Research*, **25**, 529–536.

Teodosio, C., Garcia-Montero, A.C., Jara-Acevedo, M., Sanchez-Munoz, L., varez-Twose, I., Nunez, R., Schwartz, L.B., Walls, A.F., Escribano, L. & Orfao, A. (2010) Mast cells from different molecular and prognostic subtypes of systemic mastocytosis display distinct immunophenotypes. *The Journal of Allergy and Clinical Immunology*, **125**, 719–726.

Teodosio, C., Garcia-Montero, A.C., Jara-Acevedo, M., Sanchez-Munoz, L., Pedreira, C.E., varez-Twose, I., Matarraz, S., Morgado, J.M., Barcena, P., Matito, A., Mayado, A., Sanchez, M.L., ez-Campelo, M., Escribano, L. & Orfao, A. (2013) Gene expression profile of highly purified bone marrow mast cells in systemic mastocytosis. *The Journal of Allergy and Clinical Immunology*, **131**, 1213–1224, 1224.

Teodosio, C., Mayado, A., Sanchez-Munoz, L., Morgado, J.M., Jara-Acevedo, M., varez-Twose, I., Garcia-Montero, A.C., Matito, A., Caldas, C., Escribano, L. & Orfao, A. (2015) The immunophenotype of mast cells and its utility in the diagnostic work-up of systemic mastocytosis. *Journal of Leukocyte Biology*, **97**, 49–59.

Valent, P., Akin, C., Escribano, L., Fodinger, M., Hartmann, K., Brockow, K., Castells, M., Sperr, W.R., Kluin-Nelemans, H.C., Hamdy, N.A., Lortholary, O., Robyn, J., vanDoormaal, J., Sotlar, K., Hauswirth, A.W., Arock, M., Hermine, O., Hellmann, A., Triggiani, M., Niedoszytko, M., Schwartz, L.B., Orfao, A., Horny, H.P. & Metcalfe, D.D. (2007) Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. *European Journal of Clinical Investigation*, **37**, 435–453.

Valent, P., Agis, H., Sperr, W., Sillaber, C. & Horny, H.P. (2008) Diagnostic and prognostic value of new biochemical and immunohistochemical parameters in chronic myeloid leukemia. *Leukemia and Lymphoma*, **49**, 635–638.

Valent, P., Akin, C., Arock, M., Brockow, K., Butterfield, J.H., Carter, M.C., Castells, M., Escribano, L., Hartmann, K., Lieberman, P., Nedoszytko, B., Orfao, A., Schwartz, L.B., Sotlar, K., Sperr, W.R., Triggiani, M., Valenta, R., Horny, H.P. & Metcalfe, D.D. (2011) Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *International Archives of Allergy and Immunology*, **157**, 215–225.