The clinical value of the determination of total tryptase

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INTRODUCTION

Human mast cells are derived from a haematopoietic precursor which is present in both bone marrow and peripheral blood and in umbilical cord blood[1-5], and this precursor expresses the surface antigens CD34, CD13 and CD117, but not the high-affinity receptor for IgE (FcεRI)[4,6-8]. Mast cell precursors migrate from the bone marrow to the blood and from there pass into the tissues where they mature and differentiate, acquiring the morphological, immunophenotypic and functional characteristics of the tissue where they are located, at the same time as retaining their proliferative capacity[9,10].

On activation, mast cells produce and release a large number of biologically active mediators. Some of these, such as proteases, are found preformed in the granules, while others, such as lipid mediators, are synthesized "de novo" after a necessary stimulus.

Mast cells are found in virtually all tissue types[11], particularly those which are ports of entry for various pathogens, such as mucous membranes, dermis or blood vessels, and this is believed to explain their role in innate immunity[12]. Mast cells similarly have a key role in defence against parasites[13,14] and possibly against some bacteria[15].

Mast cells are involved in responses related to immediate hypersensitivity such as asthma, rhinitis, urticaria and anaphylaxis[16]. It has recently been suggested that these cells have a role in the early phases of autoimmune diseases such as rheumatoid arthritis (reviewed in reference 17).

Tryptases are proteases located in mast cell granules and in small quantities in basophils, as demonstrated by ELISA[18] and flow cytometry[19]. The determination of tryptase values in serum has proved to be useful as a marker of mast cell activation in anaphylaxis and, more recently, as a reliable indicator of the total mast cell burden in mastocytosis[20-24].

This section will examine the biology of tryptases and their function, the techniques available for their quantification in various fluids and finally the clinical value of tryptase determination in allergic disease, anaphylaxis, mastocytosis and other blood disorders.

BIOLOGY OF HUMAN TRYPTASES

Trypsin-like activity was first described in mast cells in 1960 using histoenzymatic techniques[25]. The same activity was demonstrated in 1981 in human lung tissue mast cells[26]; the enzyme was isolated with approximately 90% purity and was called tryptase[27]. Tryptases (EC 3.4.21.59) are tetrameric cationic proteins which form a macromolecular complex with heparin[27]. Their ring-like crystalline structure was established in 1998[28].
Four genes have been identified which code for human tryptases[29,30]. Two of these, a and b, are located on chromosome 16 and are encoded by six exons. There are two predominant forms of α-tryptase (αI and αII) and three of β-tryptase (βI, βII, and βIII)[31]. The genes for the δ-tryptases[32] and the γ-tryptases[33] are also located on

chromosome 16. The product of the gene (or genes) for β-tryptase is autoprocessed in vitro, via an autocatalytic process at acid pH and in the presence of heparin, from β-protryptase to β-pro'tryptase and finally to mature β-tryptase by a dipeptidase[34]. Mature β-tryptase is stored in the secretory granules of the mast cell as an enzymatically active tetramer forming a complex with heparin. After activation, mast cell degranulation gives rise to the release of the tryptase/proteoglycan complex. By contrast, the generation in vitro of α-tryptase gives rise to the formation of tetramers which are enzymatically inactive[35-37].

β-Tryptase, which accumulates in the secretory granules of mast cells and is released during the process of exocytosis[38], increases in clinical situations associated with massive mast cell degranulation. As regards α-tryptase, recent studies appear to suggest that it is released constitutively to plasma[22] and its normal values have been determined at between 1 and 11.5 ng/mL. The α-tryptase values increase, as will be described below, in a high percentage of patients with systemic mastocytosis.

The functions of tryptases in vivo are not known precisely. In vitro studies suggest that they take part in the inactivation of fibrinogen and the inhibition of fibrinogenesis[39], the activation of collagenase in synovial cells[40], the inactivation of some neuropeptides with bronchodilating action such as VIP[41,42], the stimulation of fibroblast proliferation[43] and of the synthesis of mRNA by procollagen in culture[44] and chemotactic activity by eosinophils[45], among others.

Since tryptases occur almost exclusively in mast cells (human basophils contain only a small quantity[18]) and their half-life is longer than that of histamine, these proteases are sensitive and specific markers of mast cell degranulation in situ.

During anaphylactic reactions, there is a variable increase in serum levels of β tryptase; this increase is detected after a few minutes and reaches its peak 1-2 hours later. The increase in total tryptase (α and β tryptase) in systemic mastocytosis has been linked to the total mast cell burden[22,38]. An increase in tryptase has also been demonstrated in the bronchoalveolar lavage of asthma patients[46] and in the nasal secretions of patients with allergic rhinitis following challenge with the allergen responsible[47].

Genotypic studies have described a deficit in the gene for - tryptase in 40% of Caucasians[48]. A significant reduction in serum tryptase values was not initially detected in either normal subjects[24] or patients with systemic mastocytosis[19]. However, a recent study[49] of the βα genotype of tryptase appears to suggest that the haplotype has an effect on total tryptase values, such that they are significantly higher for the βα/βα than for the ββ/ββ haplotype; the same study detected higher tryptase values in women.

METHODS FOR QUANTIFYING TRYPTASES IN BLOOD OR OTHER FLUIDS

Immunoassay techniques are available for the quantification of tryptases owing to the development of various monoclonal antibodies, the most important of which are G5, B12 and G4. The sensitivity of G5 antibody for recognizing βII-tryptase has been described as 10 times greater than for αI-protryptase[22]. B12 and G4 monoclonal antibodies, however, recognize both tryptases (α and β) with the same affinity. It is therefore accepted that immunoassays that use the G5 antibody detect β-tryptase, while those that use B12 and G4 detect α-pro and β-tryptases, i.e. total tryptase (reviewed in references 24, 50, 51, and 52).

The initial assay used for the quantification of tryptase used G5 antibody (selective for β-tryptase) for capture and a polyclonal goat antibody for detection. The lower detection limit of this technique was determined at 2.5 ng/mL and its clinical value lay almost exclusively in the demonstration of raised circulating tryptase in patients suffering from anaphylaxis[20].

A new immunoassay was developed in 1991 which used G5 antibody for capture and G4 antibody for detection; G4 antibody recognizes both α and β tryptase. The lower detection limit in serum with this new method was ≈1 ng/mL and high values (≥1 ng/mL) were found only in cases of anaphylaxis with haemodynamic impairment[53].

Finally, a new ELISA technique was developed in 1994 using for capture mouse monoclonal antibody with isotype IgG1, known as B12, capable of detecting tryptase in plasma and serum; while G4 monoclonal antibody mentioned above was used for detection[50]. This technique significantly increased the sensitivity of the quantification of tryptases in serum or plasma with a detection limit in the region of ≈ 0.5 ng/mL. Using this method, basal tryptase is detected in virtually all normal individuals with a mean of 5 ng/mL and a range of 1 to 15 ng/mL[50].

Recent studies suggest that the serum or plasma values of tryptase precursors in normal subjects reflect total body mast cell burden, whereas mature tryptase values (technique using G5 antibody) are associated with the release of tryptase secondary to mast cell activation[24,51,52].

Currently, the only commercial technique available for the quantification of tryptase is Phadia's ImmunoCAP™ system which determines total tryptase values. Values can be determined in any type of biological fluid, in the supernatant of activated cells and in lysed cells. Figure 1 shows a diagram of the principles on which the technique is based.

All comments made from this point on will be based on results obtained using this technique.

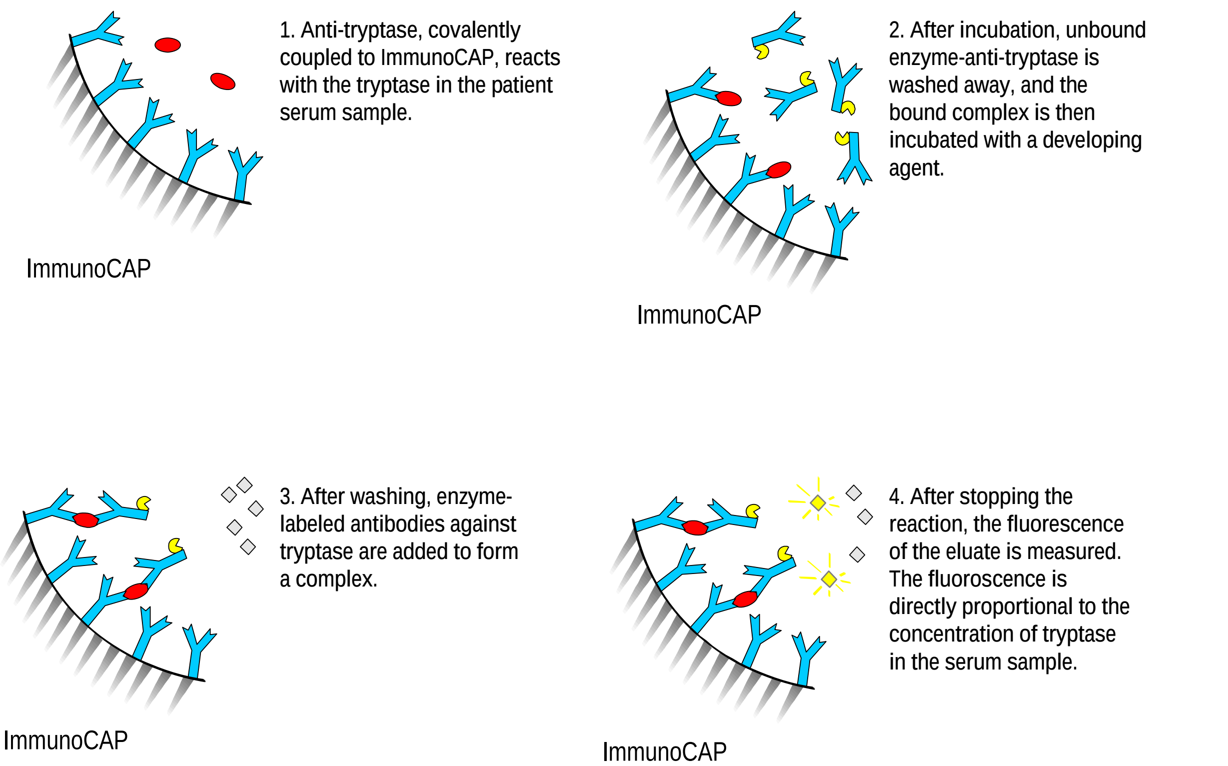

Fig. 1: Determination of tryptase

Conditions for the extraction and storage of samples for the detection of tryptase

It is essential that the samples are collected appropriately and at the appropriate time. Furthermore, if the determination will not be performed on the day of extraction, the samples should be stored correctly to prevent the degradation of proteins. In cases where the acute release of mast cell mediators is suspected, including anaphylaxis, the samples must be obtained between 30 minutes and 3 hours after the episode. It is very helpful if patients with a proven risk of mast cell degranulation carry a medical report with them stating that they may develop this type of reaction, as well as the need for correct treatment and for the collection of a serum sample which should be stored appropriately.

Samples of plasma, serum or other body fluids which may contain red cells must be centrifuged and the serum, plasma or other fluid separated into aliquots to be stored at a temperature of –20 °C until the test is performed. Other samples, such as fluid from skin blisters not containing red cells, may be frozen directly.

It is essential, for a correct interpretation of the results, to have a data collection form in which all necessary information is included. Table 1 gives an example.

Table 1: Data necessary for a correct interpretation of total tryptase

- The patient's details
- The date and time of collection
- Principal diagnosis or suspected diagnosis
- Clinical reasons for the request
- Time from the release episode (if there was one) to when the sample was obtained
- Presence or absence of signs and symptoms suggesting release of mast cell mediators
  - Flush
  - Urticaria, angioedema
  - Respiratory difficulty
  - Tachycardia
  - Hypotension
  - Syncope
  - Cardiac arrest
- Trigger or triggers (if any)
  - Physical agents such as excessive cold or heat
  - Stress and irritability (particularly in mastocytosis)
  - Drugs: aspirin and other non-steroidal anti-inflammatories, morphine and derivatives (codeine, dolantin, fentanil, etc.), cough suppressants (dextromethorphan), various antibiotics, inducers used in general anaesthesia, muscle relaxants
  - Venom (mosquitoes, wasps, bees, etc.)
- Where applicable, treatment used and respons

Controls

The selection of controls for laboratory tests is complex in some cases and the determination of tryptase is a clear example. To establish normal tryptase values, the serum or plasma used must belong to subjects who are known with relative certainty not to be suffering from any disease potentially associated with a release of mast cell mediators or, obviously, mastocytosis. In other words, subjects with a clinical history of confirmed atopic allergy or disease, including acute or chronic urticaria,angioedema, atopic dermatitis, adverse drug reactions, patients receiving immunotherapy, etc. should be excluded in principle. Individuals who have received treatment in the previous 24 hours with drugs which may induce mast cell mediator release and, of course, all those with infections, inflammatory disorders or undergoing surgery should also be excluded. Total IgE should be determined simultaneously with tryptase in all cases and samples with raised total IgE should not be used as controls.

It is possible, for all the above reasons, that the values that are currently regarded as normal may need to be revised in the future using a large number of controls so that reliable statistical studies can be carried out. This is particularly important for determining the upper normal limit which, with small variations at each laboratory, has been set at 11.5 ng/mL.

CLINICAL CONDITIONS WHICH MAY INVOLVE RAISED TRYPTASE

The determination of tryptase by the ImmunoCAP™ method is not currently considered a usual technique in the diagnosis or treatment of allergic disease; its clinical value has therefore not been clearly established nor is there any consensus on the diseases in which its quantification would be useful. Its use is currently limited, generally speaking, to studying and monitoring forms of mastocytosis and, in some cases, to anaphylactic shock with haemodynamic impairment. Table 2 summarizes possible indications for the determination of total tryptase.

Anaphylaxis

Anaphylaxis is defined as a potentially fatal systemic reaction which may flare up suddenly and whose symptoms may range from mild exanthem to vascular collapse (reviewed in reference 54). The incidence of anaphylaxis, with loss of consciousness or vascular collapse, has been estimated at 1 case per 10,000 inhabitants per year in the USA (reviewed in reference 54). Anaphylaxis may be mediated by IgE (e.g. anaphylaxis triggered by foods, allergens, Hymenoptera venom, etc.) or by other mechanisms (reviewed in reference 54).

Severe anaphylaxis, particularly when accompanied by vascular collapse, was one of the first clinical conditions in which raised β-tryptase values related to mast cell activation followed by a massive release of mediators were studied and detected. Tryptase should increase, at least in theory, in any situation of severe anaphylaxis, regardless of whether it is mediated by IgE, provided that activation of mast cells with release of their mediators has occurred (reviewed in reference 51). It has been suggested that other pathogenic mechanisms, such as basophil activation or involvement of the complement, should be considered in reactions clinically suggesting severe anaphylaxis with normal tryptase values[51].

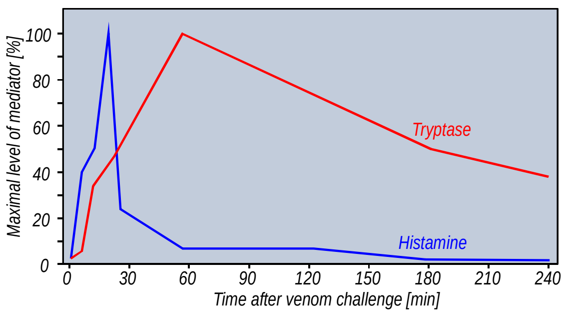

Fig. 2: Course of tryptase (grey) and histamine (black) after an anaphylactic reaction secondary to a Hymenoptera sting (Courtesy of Phadia).

Table 2: Possible indications for the determination of total tryptase
1. In serum or plasma 1.1 Clinical value established • Mastocytosis in adults - Diagnosis - Follow-up - Control of the acute release of mediators in forms of mastocytosis associated with anaphylaxis - Control of the response to the treatment of mediator release - Control of cytoreducing treatment in cases which might require it • Mastocytosis in children - At the time of diagnosis - In patients with severe symptoms of release DCM UP with repeated pictures of skin blistering or flushing. Monitoring of patients with raised basal tryptase • Anaphylaxis of any aetiology - In the acute phase - Under basal conditions* • Blood disorders other than mastocytosis - Acute myeloblastic leukaemia** - Myelodysplastic syndromes** - Primary hypereosinophilic syndromes*** 1.2. Clinical value not established • Preoperative studies in patients at risk**** - History of adverse reactions to general anaesthesia - Patients with a history of atopic or allergic disease - Patients with mastocytosis • Assessment of the severity of allergic disease • Assessment of the course and/or response to treatment in allergic disease • Study of serious reactions during provocation tests with allergens or drugs. Study of adverse reactions during immunotherapy • In forensic medicine - To rule out possible anaphylaxis during surgery - Post mortem determination of tryptase to rule out anaphylaxis as a cause of death 2. In other fluids • In allergic disease (clinical value not established) - Bronchoalveolar lavage - Nasal exudate - Tears • In forms of mastocytosis - In ascitic fluid in aggressive systemic forms of mastocytosis - In the contents of skin blisters from paediatric patients with diffuse cutaneous mastocytosis or urticaria pigmentosa 3. In vitro studies • Study of mast cell activation - In isolated mast cells - In mast cell cultures *If basal tryptase is raised, exclude severe allergic disease and, if it is not raised, exclude mastocytosis associated with anaphylaxis. **If basal tryptase is raised, carry out serial tests to monitor the response to the treatment. Useful for monitoring minimal residual disease. ***If basal tryptase is raised, exclude an association with abnormal mast cells (abnormal morphology and expression of CD25). ****Apply specific anaesthesia protocols. Check tryptase values during and after surgery.

Anaphylaxis due to Hymenoptera venom

During anaphylaxis induced by an insect or Hymenoptera sting, the serum levels of β tryptase reach a peak in the circulation after between 15 and 120 minutes and take 150 to 180 minutes to return to basal values; by contrast, levels of histamine reach a peak after 15 minutes and return to normal in 15 to 30 minutes[21,38]. Quantification of tryptase can thus confirm the diagnosis of anaphylaxis, even if the sample was collected several hours after the acute picture (Fig. 2).

As regards anaphylaxis due to a Hymenoptera sting, patients with raised basal tryptase have been described as having a significantly higher risk of severe anaphylactic reactions[55,56]. It therefore seems reasonable to include the determination of tryptase, both in the acute phase and under basal conditions, in the study of these patients; this would allow more precise data to be obtained on the degree of mast cell activation and, possibly, the severity of the response to the venom. Furthermore, the possibility of mastocytosis with or without skin lesions should be excluded specifically in patients with raised basal tryptase regardless of whether specific IgE antibodies to the venom are present and regardless of the result of skin tests (see below).

The measurement of tryptase during immunotherapy, particularly if adverse effects appear, should similarly be regarded as a usual indication and could perhaps suggest whether a patient undergoing immunotherapy is at risk of serious adverse effects. Particular attention should also be paid in these cases to patients with raised basal tryptase.

It is only by conducting prospective studies with sufficient cases that the true value of tryptase for assessing the severity of a reaction to venom can be determined, along with its ability to predict the behaviour of a given patient during future provocations or specific immunotherapy.

Anaphylaxis in general anaesthesia

Many of the drugs used in general anaesthesia are capable of causing the release of mediators by mast cells and basophils; these include inducers, muscle relaxants and morphines[57-61]. The frequency of anaphylaxis in the perioperative period has been estimated at between 1 in 10,000 and 1 in 30,000 (reviewed in reference 62). Neuromuscular blockers were the drugs involved most frequently (69%) followed by latex (12%), antibiotics (8%), hypnotics (3.7%), morphines (1.4%) and colloids (2.7%)[62]. The same study described 23% of anaphylactic reactions as grade II, 63% as grade III and 4.4% as grade IV. Furthermore, the authors confirmed a predominance of anaphylaxis in women as compared with men (2.7/1).

The neuromuscular blockers with the highest risk are succinylcholine and rocuronium[63-65] and reactions are least frequent with anaesthesia inducers such as etomidate or hypnotics such as thiopental, propofol or midazolam[66-68] (reviewed in reference 62).

Patients with a history of adverse reactions to any of the drugs mentioned or those considered to be at high risk must be examined carefully before undergoing general anaesthesia according to established protocols (reviewed in reference 69). Furthermore, in these cases the initial clinical examination should include a determination of basal tryptase and if this is raised, the possibility of mastocytosis (see below) or serious allergic disease must be ruled out; in this last case, however, a possible link has not been established between the severity of allergy and total tryptase values.

Although there is no consensus on this point, patients confirmed as being at high risk for general anaesthesia could be premedicated with H1 and H2 antihistamines and prednisone; it has, however, been suggested that this treatment may mask the initial phases of anaphylaxis. Anaesthesia should be carried out using inducers and muscle relaxants with a low capacity for inducing mast cell and basophil degranulation. The value of determining tryptase has not been established in the study of adverse reactions during anaesthesia and only prospective studies, measuring tryptase before, during and after surgery, would provide this information.

Patients with mastocytosis constitute a high-risk group, although the exact percentage of anaesthesia complications in this group is not known, nor are there reliable parameters for determining which patients are more likely to develop adverse reactions during anaesthesia. Serious reactions and even death have been described[70-77], and various general anaesthesia protocols have been proposed to prevent this type of complication[78-85]. The protocols currently used in the Mastocytosis Unit of Ramón y Cajal Hospital include premedication with H1 and H2 antihistamines, with or without prednisone, and antileukotrienes (Mariana Castells, personal communication, October 2002), vecuronium as a neuromuscular blocker and etomidate as an inducer.

TRYPTASE AND ALLERGY

Neither the indications nor the possible advantages of tryptase determination in atopic or allergic disease have been established. There are isolated studies which suggest that it is useful for assessing the response to treatment in atopic dermatitis[86] and in allergic rinitis[87]. It has also been suggested that it could be an indirect marker of inflammation in asthma[88]. Raised tryptase values have been found in bronchoalveolar lavage samples from asthmatic children[89] and patients with bronchiolitis obliterans[90], and in nasal lavage fluid from patients with allergic rhinitis[91].

The role of tryptase in assessing the severity of allergy is not known. Apart from in anaphylaxis associated with vascular collapse, raised tryptase values have been found in a variable percentage of patients with acute urticaria, urticaria/angioedema and anisakiasis, etc. (Concepción Sánchez, personal communication, September 2002). Prospective clinical studies including a sufficient number of cases are, however, essential to establish its real clinical value in allergic disease.

VALUE OF THE DETERMINATION OF TRYPTASE IN FORENSIC MEDICINE

The post-mortem use of tryptase determination and its possible applications in forensic medicine for diagnosing death induced by anaphylaxis are very controversial. Raised tryptase has been described in cases in which death appeared to be due to anaphylaxis secondary to various causal agents such as Hymenoptera stings, foods or intravenous medication, etc.[92-94]. There is, however, no consensus on the real value of tryptase determination post mortem, due essentially to the fact that raised tryptase values have been found associated with the time from death to the collection of the sample and a link has also been suggested between increases in this protease and arteriosclerosis, chest traumas and cell lysis in the absence of anaphylaxis[93,94] (reviewed in reference 51). It has been suggested that the combined study of total tryptase and specific IgE antibodies to suspected allergens could increase the sensitivity and specificity[94]. To summarize, the current role of tryptase determination post mortem for establishing a diagnosis of anaphylaxis ante mortem has not been clearly established. To determine precisely its possible role from a legal point of view, complex prospective studies are necessary which would include the determination of tryptase in blood, and possibly of total IgE and specific IgE, in persons who have died from causes unrelated to anaphylaxis. It would be necessary, for this purpose, to measure tryptase at the time of death, if possible, and at certain intervals (for example between 1 and 24 hours post mortem). The appropriate controls could thus be established and the real value of tryptase when death related to anaphylaxis is suspected could be examined.

VALUE OF TRYPTASE IN THE STUDY OF MASTOCYTOSIS

Mastocytosis is a heterogeneous group of diseases characterized by mast cell proliferation in various tissues such as skin, bone marrow, the digestive tract and bone, among others. There are various forms of mastocytosis as regards age of appearance (paediatric and adult forms), the number of tissue types affected (pure cutaneous forms and systemic forms) and clinical behaviour (indolent or aggressive). The signs and symptoms that appear in mastocytosis may be due to the release of mast cell mediators, the infiltration of various tissues by mast cells or both. There is not always a direct relation between total mast cell mass and the symptoms of release (reviewed in reference 85); some patients may thus remain asymptomatic, while others with the same form of the disease suffer severe symptoms related to mediator release.

Mastocytosis is easier to diagnose when there is a skin lesion, which applies in approximately 80% of cases. To establish whether the disease is localized (pure cutaneous forms, benign solitary mastocytoma, etc.) or systemic, direct or indirect methods need to be used to determine whether the bone marrow or other organs are affected. A bone marrow study must include cytology[95], immunohistochemical detection of mast cell tryptase[96], immunophenotypic characterization of bone marrow mast cells by flow cytometry to detect the possible existence of an aberrant immunophenotype[97-100] and finally a PCR study of activating mutations of the c-kit gene (reviewed in reference 85). Along with the determination of total tryptase and various clinical and analytical parameters, these methods have formed the basis of the new consensus classification for mastocytosis drawn up by a group of experts in Vienna in September 2000[101]. Tables 3 and 4 show the diagnostic criteria and the classification. The classification established major and minor diagnostic criteria, including a serum tryptase value above 20 ng/mL.

In adult mastocytosis, total tryptase is normal in pure cutaneous forms, moderately raised in the majority of indolent forms, and above 200 ng/mL in aggressive forms and in mast cell leukaemias. In indolent systemic mastocytosis, the most frequent clinical form in adults, tryptase is usually normal in the initial stages of the disease, it increases during the first few years until it reaches approximately 35 ng/mL and may remain at similar values for more than 10 years in a large percentage of patients (Luis Escribano, unpublished data) suggesting that the total mast cell burden remains stable.

Table 3: Diagnostic criteria in systemic mastocytosis*

A. Major criteria
1. Histological/immunohistochemical alterations: mast cell aggregates containing more than 15 mast cells

B. Minor criteria
1. Cytological alterations: > 25% of morphologically abnormal mast cells
2. Detection of c-kit mutations on codon 816
3. Immunophenotypic alterations: expression of CD25 (± CD2) in mast cells from bone marrow, peripheral blood or other organs
4. Total serum tryptase levels persistently >20 ng/mL (not applicable if there is a related blood disorder or evidence of acute mast cell release)

C. Diagnosis of systemic mastocytosis
a. 1 major criterion + 1 minor criterion
b. 3 minor criteria

*Taken from reference 101.

Table 4: Consensus classification of mastocytosis*

Cutaneous mastocytosis
Variants:
- Urticaria pigmentosa (UP)
  - Subvariants:
    - Typical (maculopapular UP)
    - Plaques
    - Nodular
    - Telangiectasia macularis eruptiva perstans
    - Diffuse cutaneous mastocytosis
- Cutaneous mastocytoma

Indolent systemic mastocytosis
- Provisional subvariant:
  - Bone marrow mastocytosis (isolated)
- Provisional subvariant:
  - Smouldering systemic mastocytosis

Systemic mastocytosis with other associated clonal blood disorder

Aggressive systemic mastocytosis
- Subvariant:
  - Lymphadenopathic form with eosinophilia

Mast cell leukaemia (ML)
- Subvariants:
  - Classic (≥10% MC in peripheral blood)
  - Aleukaemic variant (<10% MC in peripheral blood)

Mast cell sarcoma

*For further details, see reference 101.

Mastocytosis associated with recurrent anaphylaxis, with or without a precise aetiology, is a relatively frequent form of the disease, with a prevalence of 27% (9 out of 30 cases) at the Laboratory of Allergic Diseases (NIAID/NIH) which is a reference centre for both mastocytosis and idiopathic anaphylaxis[102] and 7.9% (12 out of 151 cases of adult mastocytosis) at the Mastocytosis Unit of Ramón y Cajal Hospital (REMA, unpublished data). In these cases, total tryptase remains high once the acute episode has disappeared, and this elevation is of particular value for differential diagnosis for excluding mastocytosis. In our experience, forms associated with anaphylaxis due to Hymenoptera stings are the most frequent. It should be kept in mind that the presence of specific IgE antibodies to the venom does not exclude a diagnosis of mastocytosis in these patients. In a recent study of 259 patients with Hymenoptera venom allergy, raised basal tryptase values were found in 19 cases and mastocytosis with cutaneous involvement was demonstrated in 3 of these[56]; no specific studies were conducted, however, to rule out mastocytosis in those patients with raised tryptase and without skin lesions.

Immunotherapy has proved to be effective in protecting some patients in mastocytosis associated with anaphylaxis due to Hymenoptera venom[103], and ineffective in other cases[104]. It was not specified, however, whether or not the patients had specific IgE antibodies. It is not advisable for immunotherapy to be used in patients in whom the presence of specific IgE antibodies to the corresponding venom cannot be demonstrated.

No prospective studies have been published into the use of tryptase in paediatric mastocytosis. Our experience suggests that tryptase determination at the time of the diagnosis can predict the severity of symptoms in both urticaria pigmentosa and diffuse cutaneous mastocytosis. Accordingly it is rare for children with normal basal tryptase to suffer severe symptoms related to mast cell mediator release while, by contrast, those with raised tryptase frequently present with severe pruritus and flushing requiring specific treatment (Spanish Network on Mastocytosis, unpublished data). Furthermore, in paediatric forms of diffuse cutaneous mastocytosis with anaphylaxis associated with vascular collapse, the most severe paediatric form, serial determination of tryptase has a direct influence on therapeutic decisions and monitoring of the response[105].

To summarize, the experience of the Mastocytosis Unit of Ramón y Cajal Hospital suggests that the determination of total tryptase in mastocytosis is a major advance in the diagnosis, differential diagnosis and follow-up and monitoring of the response to cytoreducing treatments in this disease. Furthermore, owing to its sensitivity for reflecting total mast cell burden, except in the presence of massive mast cell mediator release or some blood disorders (see below), the course can be monitored closely without the need to repeat bone marrow studies, and this has an obvious influence on patient quality of life.

VALUE OF TRYPTASE IN OTHER BLOOD DISORDERS

Raised tryptase to more than 20 ng/mL has been found in approximately 30% of patients with acute myeloblastic leukaemias[106] and in a similar percentage of myelodysplastic syndromes[107]. There is no doubt that this finding is important from a practical point of view, since in acute leukaemias with raised tryptase at the time of the diagnosis, the response to chemotherapy is accompanied by a reduction in tryptase which normalizes when a complete remission is achieved, and rises during relapses[106]. Tryptase is therefore a marker of the myeloid line and also a useful and simple parameter for monitoring the response and minimal residual disease in acute myeloblastic leukaemias.

Raised tryptase has also been described in a group of idiopathic hypereosinophilic syndromes characterized by the presence of abnormal mast cells in bone marrow (atypical morphology and aberrant expression of CD25 antigen)[108], aggressive clinical pictures, the presence of the FIP1L1-PDGFRA fusion gene but not kit activating mutation, and response to treatment with the tyrosine kinase inhibitor Glivec®[108].

Summary

The determination of total tryptase by the technique currently available provides us with information, at least in theory, on both mast cell activation, associated with mature β tryptase, and the mast cell burden in the body, associated with α-protryptase. Its clinical value has been demonstrated up to now in various pathological conditions such as anaphylaxis, mastocytosis and some myeloid blood disorders. It is possible, however, that in the future the determination of these proteases will enable a more appropriate assessment of the severity of allergic disease and probably of the response to its treatment. It is therefore necessary to carry out prospective studies including sufficient cases to obtain statistically valid results.

Acknowledgements

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