

WHITE PAPER

# The Serum Tryptase Test: Use in Clinical Hematology

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Within the last decade, various molecular markers have been introduced in clinical hematology. The majority of these markers are indicators of specific gene defects and thus are of importance in diagnosis, prognostication and in the follow-up (minimal residual disease monitoring). Apart from these specific markers, serologic parameters are also considered to be of importance in the diagnosis and prognostication of hematologic neoplasms.

Of all markers developed in the recent past, serum tryptase appears to be a most reliable and most informative serologic biomarker of myeloid neoplasms. Various myeloid disorders and neoplasms, including systemic mastocytosis, myelodysplastic syndromes, myeloproliferative neoplasms, acute myeloid leukemias, chronic myeloid leukemia and chronic eosinophilic leukemia can present with elevated tryptase levels. Recent data suggest that the serum tryptase level is of diagnostic and/or prognostic significance in these patients.

Since 2001, a clearly elevated serum tryptase level (>20 ng/ml) is a minor criterion of systemic mastocytosis (SM) as defined by the World Health Organization (WHO). Moreover, tryptase is an important prognostic marker in advanced SM. In chronic myeloid leukemia (CML), an elevated serum tryptase level (>15 ng/ml) at diagnosis is of predictive value as a prognostic biomarker. In the present article, we focus on the clinical value of tryptase measurement in daily practice and provide recommendations for use of this biomarker in clinical hematology.

## List of Abbreviations

AML	Acute Myeloid Leukemia
ANF	Atrial Natriuretic Factor
ASM	Aggressive Systemic Mastocytosis
CEL	Chronic Eosinophilic Leukemia
CML	Chronic Myeloid Leukemia
CMML	Chronic Myelomonocytic Leukemia
ICUS	Idiopathic Cytopenia of Unknown Significance
ISM	Indolent Systemic Mastocytosis
MC	Mast Cell
MCAS	Mast Cell Activation Syndrome
MCL	Mast Cell Leukemia
MDS	Myelodysplastic Syndrome
MML	Myelomastocytic Leukemia
MPN	Myeloproliferative Neoplasm
SM	Systemic Mastocytosis
SM-AHN	Systemic Mastocytosis with associated Hematologic Neoplasm
SM-AML	Systemic Mastocytosis with associated Acute Myeloid Leukemia
VIP	Vasoactive Intestinal Peptide

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## Introduction

Tryptase is a term collectively used for a family of trypsin-like serine proteases that are encoded at 16p13.3, the short arm of chromosome 16.<sup>1-4</sup> This locus includes several genes, i.e. *TPSG1*, *TPSB2*, *TPSAB1*, *TPSD1*, and *TPSE1* encoding for  $\alpha$ - and  $\beta$ - tryptase, the major protein product of human mast cells (MCs).<sup>5-8</sup> Tryptase can also be detected in immature blood basophils.<sup>9-11</sup> The enzyme is produced and released from MCs independent of the tissue location of these cells, their maturation stage, or the subtype of MCs.<sup>9</sup> Whereas MCs produce and release the pro-alpha form of tryptase constantly, the beta-form is primarily stored in MC granules and is released upon activation, e.g. during an anaphylactic reaction.<sup>8</sup>

Tryptases interact with a limited number of known natural substrates but these enzymes also have a wide range of biological activities. In fact, tryptases are involved in the regulation of cell proliferation and growth, processing of (pro)hormones, activation of fibrinolytic enzymes, and degradation of plasma and matrix molecules. Likewise, tryptase appears to be a most potent mitogen for various mesenchymal cells, including fibroblasts and endothelial cells.<sup>12-14</sup> In addition, tryptase reportedly degrades fibrinogen and activates pro-urokinase.<sup>15, 16</sup> Other biologic effects include the cleavage of vasoactive intestinal peptide (VIP)<sup>17</sup> and degradation of pre-atrial natriuretic factor (ANF) into its active form.<sup>18</sup> The enzymatic activity of  $\beta$ -tryptase depends on environmental factors.

Optimal conditions and factors appear to be present within MC secretory granules, the primary site of enzyme expression and storage. Tetramer formation *in vitro* is optimal at low pH (6.0), a ionic strength equivalent of 160 mM NaCl, and a temperature-range of 22–37 °C.<sup>19, 20</sup> At neutral pH, the enzymatically active (tetrameric) form of the enzyme is stabilized by heparin.<sup>19-21</sup>

Several assays measuring tryptase have been developed during the last two decades.<sup>22</sup> The most widely used assay, a fluoroimmuno-enzyme assay, detects all major tryptase species (alpha and beta forms and all pre-forms of the enzyme).<sup>23</sup> This assay is commercially available and is a simple, reliable and highly reproducible assay. There are no pre-analytical caveats and the serum samples can be shipped or stored before measurements.

An elevation of serum tryptase has a number of diagnostic implications.<sup>9</sup> In anaphylactic reactions, a rapid increase of tryptase levels above baseline can occur.<sup>24, 25</sup> After the event, it takes several hours to days until the tryptase level returns to baseline.

Therefore, comparing the basal serum tryptase level measured in the symptom-free interval with that measured during an anaphylaxis-event can confirm the presence of a mast cell activation syndrome (MCAS).<sup>24, 25</sup> Moreover, there is a positive correlation between the serum tryptase level and the severity of the anaphylactic reaction.<sup>11</sup> MCAS is a severe condition characterized by episodic symptoms of flushing, hypotension and anaphylaxis. In these patients, the event-related increase in serum tryptase above a certain limit is indicative of the involvement of MCs and is thus a reliable diagnostic criterion of MCAS.<sup>26-28</sup>

A persistent elevation of serum tryptase levels is detectable in a variety of conditions and disorders. Likewise, as mentioned before, elevated serum tryptase levels are detectable in various myeloid neoplasms, whereas almost all patients with lymphoid neoplasms have a normal tryptase level.<sup>28-30</sup> Elevated tryptase is found in about 90 % of patients with SM, 37 % with AML, 34 % with CML, and 24 % with MDS.<sup>24, 25</sup> The highest tryptase levels, often exceeding 1000 ng/ml, are found in patients with advanced MC neoplasms and in those with core binding factor leukemias.<sup>29-33</sup> Serum tryptase levels are below 20 ng/ml in a majority of the cases presenting with reactive leukocytosis/thrombocytosis or idiopathic cytopenia.<sup>29</sup> Among patients with non-hematologic disorders, a few cases with end stage renal failure or helminth infections were found to have slightly elevated serum tryptase concentrations.<sup>29</sup> An important differential diagnosis in all these cases is hereditary (familial) (hyper) tryptasemia (HAT) where tryptase levels are commonly elevated (>11.4 ng/ml) compared to healthy controls without HAT.<sup>5, 34</sup>

## Tryptase Levels in Healthy Individuals

The median serum tryptase level found in healthy subjects (excluding asymptomatic HAT carriers) averages at about 5 ng/ml and ranges between <1 and 11.4 ng/ml. In more than 99 % of these healthy controls, serum tryptase levels are below 10 ng/ml.<sup>23, 29, 31, 34</sup> When adding asymptomatic HAT cases, the normal serum tryptase level ranges between <1 and 15 ng/ml.<sup>12, 29, 35</sup> Women and men slightly differ in their median serum tryptase levels, with female cohorts expressing a lower medium tryptase value compared to males.<sup>29</sup> Subjects aged <16 years also have a slightly lower serum tryptase concentration compared to adults. Thus, serum tryptase levels in healthy individuals increase with age.<sup>29, 36</sup>

For most clinicians a serum tryptase level <11.4 ng/ml is used as cut off to delineate between normal and elevated.

Sometimes even <10 ng/ml is used as a 'decision level' for further investigations. Today, we know that many healthy asymptomatic individuals with a slightly elevated tryptase have HAT. It should also be noted that heterophilic antibody-interference resulted in false-positive results on rare occasions in the original assay.<sup>36-38</sup>

Some individuals with chronic active helminth infection or renal failure can also present with a slightly increased serum tryptase level.<sup>29,39-41</sup> As mentioned before, a transient elevation of tryptase levels is a characteristic finding in cases with an anaphylactic reaction.<sup>22,24,25</sup>

### **Tryptase Levels in Non-Hematologic Disorders**

A rapid increase in serum tryptase is a typical finding in patients with substantial systemic MC activation and anaphylaxis, as typically seen in MCAS.<sup>24-27,28</sup> The term MCAS denotes a heterogeneous group in disorders characterized by episodic symptoms of hypotension and anaphylaxis, sometimes accompanied by abdominal pain and flushing.<sup>26-28,42-44</sup> Three distinct types of MCAS have been defined: in patients with primary MCAS, clonal MCs with mutated *KIT* and expression of CD25 on MCs is found; most of these cases are suffering from (systemic) mastocytosis. In secondary MCAS, patients are typically suffering from an underlying hypersensitivity disorder, such as an IgE-dependent allergy or a hypersensitivity reaction against foods or drugs.<sup>26</sup> When neither clonal MCs nor an overt allergic or other inflammatory disorders is detectable and no trigger for a hypersensitivity reaction is found the diagnosis of idiopathic MCAS is appropriate.<sup>26</sup>

On the other hand, serum tryptase levels are known to be elevated in a number of non-hematologic and non-allergic disorders. These disorders comprise hereditary alpha-tryptasemia (HAT), chronic renal failure, and chronic helminth infections.<sup>5,29,40-42</sup>

### **Hereditary Alpha-Tryptasemia (HAT)**

The tryptase locus contains four genes (*TPSG1*, *TPSB2*, *TPSAB1*, and *TPSD1*) of which only *TPSB2* and *TPSAB1* encode the secreted isoforms of tryptase.<sup>45</sup> Recently, familial cohorts with increased copy numbers of  $\alpha$ -tryptase-encoding regions resulting in an elevated basal serum tryptase level, have been described and termed hereditary alpha-tryptasemia (HAT).<sup>5,34</sup> Characteristic findings in such patients are hypersensitivity reactions to certain triggers (typical: vibration-induced) with urticaria, flushing and pruritus, gastrointestinal symptoms, connective tissue abnormalities, and symptoms suggestive of autonomic dysfunction.<sup>5,36,42-44</sup> In patients with mastocytosis and IgE-dependent allergies, the HAT status correlates with the severity of symptoms.

An association between the copy numbers of the  $\alpha$ -tryptase gene, the basal serum tryptase level, and the severity of clinical symptoms has also been described.<sup>45</sup>

### **Chronic Kidney Disease and End-Stage Kidney Disease**

Slightly elevated tryptase levels are often detected in patients with end-stage kidney disease requiring hemodialysis and tryptase levels were found to correlate with markers of chronic renal injury, including serum creatinine and proteinuria.<sup>29,39,40</sup> As published previously, in roughly 7 % of all patients with slightly elevated tryptase (above 11.4 ng/mL) a chronic kidney disease was found. Whether MCs are involved in the pathogenesis of renal damage remains uncertain.<sup>46,47</sup> However, according to literature elevated numbers of MCs were detectable in the interstitium.<sup>39,40,46,47</sup> Moreover, in the light of recent data it is tempting to speculate that some of these patients with elevated serum tryptase levels might have HAT.

### **Helminth Infections and Cardiovascular Disorders**

MCs are supposed to play a role in parasitic infections.<sup>48</sup> However, only in rare cases with helminth infection elevated serum tryptase levels were found.<sup>29,41</sup> In patient with cardiac disorders including coronary artery disease, those with poor outcome were found to have higher serum tryptase levels compared to the patients with favorable outcome.<sup>49-51</sup> Of note, the median serum tryptase levels in both groups were below 10 ng/mL.<sup>49-51</sup>

### **Tryptase Testing in Hematologic Disorders**

In most patients with reactive leukocytosis/thrombocytosis or idiopathic cytopenia, serum tryptase levels are normal or only slightly elevated (<15 ng/ml). Clearly elevated levels of tryptase (>15 ng/ml) are found in patients with myeloid neoplasms, including SM, MDS, MPN, AML, CML and CEL. In patients with lymphoid neoplasms, including lymphomas and multiple myeloma, tryptase levels are usually below 15 ng/ml.<sup>29</sup>

Normal or near normal tryptase levels (<15 ng/ml) are usually also found in patients with non-hematologic malignancies, including reactive leukocytosis/thrombocytosis or idiopathic cytopenia including patients with idiopathic cytopenia of unknown significance (ICUS).<sup>52-54</sup> Myeloid neoplasms in which patients may present with elevated tryptase, include SM, AML, MDS, chronic myelomonocytic leukemia (CMML), CML, and CEL.<sup>29,32,55-57</sup> It is of note that not all patients in these groups exhibit elevated tryptase levels and that the percentage of cases with elevated tryptase varies, depending on the type of disease. In particular, >90 % of the patients with SM have a markedly elevated serum tryptase level.

About 30-40 % of all patients with AML and 30-35 % of all patients with CML present with a serum tryptase >15 ng/ml at diagnosis.<sup>29,33,58</sup> By contrast, the incidence is much lower in patients with MPN and MDS.<sup>29,55</sup> Exceptions are patients with MPN-eo or CEL in whom neoplastic cells express the FIP1L1/PDGFR $\alpha$  fusion gene. In a majority of these cases an elevated serum tryptase level is detected.<sup>29,56,57</sup> Whether in some of the cases with SM, AML, CMML, CML, MDS, and CEL, a HAT is also present and whether the HAT status is of prognostic significance in these patients, remains at present unknown.

### Tryptase Testing in Mastocytosis

The initial development of tryptase as a diagnostic marker in clinical hematology focused on mastocytosis.<sup>31,59-61</sup> Notably, patients with SM almost always present with serum tryptase levels >20 ng/ml whereas two thirds of the patients with cutaneous mastocytosis (CM) have a tryptase concentration below 15 ng/ml.<sup>14,29,32,34,62,63</sup> The group of indolent SM (ISM) shows a broad range of tryptase levels.<sup>32,64</sup> In patients with very high serum tryptase levels (>200 ng/ml), smoldering SM (SSM) may be diagnosed.<sup>29,33,64-67</sup> Of note, a serum tryptase level >200 ng/ml is a criterion of SSM.<sup>64</sup> Almost all patients with advanced SM, i.e. aggressive SM (ASM), MC leukemia (MCL), and SM with an associated hematologic neoplasm (SM-AHN) have elevated enzyme levels, often exceeding >200 ng/ml.<sup>32,63,64</sup> Elevated serum tryptase levels are also found in patients with myelomastocytic leukemia (MML), a major differential diagnosis to MCL.<sup>65-67</sup> In contrast to ASM and MCL, tryptase levels in patients with ISM and SSM usually remain in a stable range over time, which is of particular clinical importance.<sup>29,32,66</sup> By contrast, in patients who have or progress to MCL, serum tryptase levels often increase over time, sometimes up to >1000 ng/ml.<sup>29,33,59-61</sup>

Tryptase levels >20 ng/ml has been defined as a minor diagnostic criterion of SM by the WHO.<sup>59-61</sup> At present, tryptase levels are widely used as a robust screen-parameter for patients with suspected SM.<sup>24,29,33,68-71</sup> In adults with tryptase levels >20 ng/ml the likelihood of SM is high, especially in combination with typical skin lesions. This is also the case in patients suffering from a known hymenoptera venom allergy where an elevated serum tryptase level can sometimes be measured in the symptom-free interval and may be indicative of SM even if no skin lesions are found.<sup>24,65,70</sup> In these patients, diagnostic procedures should include a bone marrow biopsy, and in most cases, such investigation will reveal SM and the *KIT* mutation D816V is often detectable.<sup>68-72</sup> An alternative (differential) diagnosis in these patients is HAT.

Serum tryptase levels vary significantly among different subsets (variants) of mastocytosis. In cases with advanced SM, tryptase is an independent prognostic marker and is therefore also used as a prognostic variable in the international prognostic scoring system for mastocytosis.<sup>29,33,73</sup>

### Tryptase Testing in AML

Several reports have shown that serum tryptase concentrations above 15 ng/ml are found in 30-40% of all patients with de novo AML.<sup>29,32,58</sup> In AML cases with elevated tryptase levels the leukemic blasts express and release the enzyme.<sup>32</sup> The percentage of tryptase-positive cases as well as the median tryptase concentration at diagnosis correlates with the subtype of AML as well as with the karyotype.<sup>32</sup> Interestingly, the highest tryptase levels were detected in patients with core binding factor AMLs.<sup>32,58</sup> In cases with acute promyelocytic leukemia a majority of patients have serum tryptase levels >20 ng/ml.<sup>32</sup> But also in the group of patients with intermediate karyotype or unfavourable karyotype a subset of patients was found to have elevated serum tryptase. In a few AML patients with elevated tryptase levels MC-lineage involvement and/or the *KIT* D816V mutation are detectable. In these patients, overt SM (SM-AML) is usually diagnosed.<sup>74-77</sup>

In patient with tryptase-positive AML the enzyme levels can be employed to monitor the disease during and after chemotherapy.<sup>58</sup> In patients who have a persistently elevated tryptase level at the time of complete hematologic remission (CR) or have a recurrent increase in tryptase during follow-up, relapses occur in a majority of the cases.<sup>58</sup> SM-AML represent an important exception.<sup>76</sup> In most of these patients, serum tryptase levels are derived from the SM component of the disease and not from AML cells. As a result, no substantial decrease in tryptase is seen during and after chemotherapy (or even after stem cell transplantation) in these patients as the SM component is usually resistant. However, persistence of elevated tryptase levels does not imply a resistance of AML to chemotherapy in SM-AML. Indeed, there are patients with SM-AML with a long-term relapse-free survival after stem cell transplantation despite the persistence of the SM-component of their disease and thus persistently elevated serum tryptase levels.

### Tryptase Testing in Ph+ CML

Basophilia is one of the most important established risk factors in CML.<sup>11</sup> These cells are the primary source of tryptase in this disorder and an elevation of tryptase can be observed in 30-40% of the patients.<sup>29,78,79</sup> About one third of the patients with chronic phase (CP) CML present with tryptase levels >15 ng/mL, whereas about 70 %

of the patients with advanced CML (accelerated or blast phase CML) present with clearly elevated tryptase levels (>15 ng/mL).<sup>29,78,79</sup> Moreover, significant differences in tryptase levels are found when comparing the prognostic risk groups of the Sokal, Hasford, and EUTOS Score, and serum tryptase levels also correlate with basophil counts in patients with CML.<sup>79</sup> In CP patients with serum tryptase >15 ng/ml the risk of progression is high, and marked differences are also seen when comparing the event-free survival in CP patients with normal or elevated serum tryptase levels.<sup>78,79</sup>

These data suggest that serum tryptase is of predictive value as a prognostic biomarker in newly diagnosed CML.<sup>80</sup> Moreover, a recent study showed that serum tryptase levels can replace basophil counts in the EUTOS score used to define the progression-risk in patients with freshly diagnosed CML.<sup>80</sup> Indeed, this modified EUTOS-T score may enhance the prognostic value of this score with regard to survival-prediction in CML patients treated with imatinib.<sup>80</sup>

#### **Tryptase Testing in Other Myeloid Neoplasms: MDS, MPN and CEL**

Serum tryptase levels are also elevated in distinct subsets of patients suffering from other myeloid neoplasms. Thus about 25 % of the patients with MDS and about 30 % of the patients with CEL have elevated enzyme levels.<sup>29</sup> In the group of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF), only a few patients exhibit elevated serum tryptase levels.

Patients exhibiting a FIP1L1/PDGFRA fusion gene product in neoplastic cells represent a defined subset of patients with CEL, MPN or a MDS/MPN overlap disease presenting with eosinophilia (MPN-eo or MPN/MDS-eo).<sup>28</sup> Clonal mast cells (CD25+) detectable in some of these cases may contribute to the elevated tryptase level. Only few of these patients have an overt associated SM (SM-CEL).<sup>80,81</sup> However, elevated tryptase levels are not restricted to MDS-eo or MPN-eo variants defined by expression of PDGFRA fusion genes.<sup>29,55</sup> MC hyperplasia, an increase in immature basophils or an increase in tryptase+ blast cells or HAT may contribute to an elevated tryptase level in such patients.<sup>29,35,82</sup>

In contrast to AML and CML, serum tryptase levels do not have a major diagnostic or prognostic impact in MDS or MPN – and therefore, we do not recommend the general use of tryptase in patients with known MDS or MPN unless eosinophilia or an increase in mast cells is seen.<sup>29,35</sup>

In these cases serum tryptase testing is of importance because some of these patients may indeed have a PDGFR- or FGFR-rearranged neoplasm or SM-AHN. Finally, in case of a very high serum tryptase levels at diagnosis enzyme levels can be employed as follow-up parameter. If this is not the case, the tryptase test should only be performed once at diagnosis in patients with MDS or MPN.

#### **Tryptase as Routine Test Parameter in Clinical Hematology**

Several reports have shown, that various groups of patients with myeloid neoplasms can present with increased tryptase levels. Since this parameter is of diagnostic and prognostic potential, many clinicians have established tryptase testing for use in daily practice, in particular for taking care of patients with SM. Employing tryptase as a screen parameter is of importance in patients with suspected mastocytosis, CEL, and other myeloid neoplasm.

Serum tryptase may be a useful non-invasive screen-parameter in patients with cytopenias, leukocytosis, or thrombocytosis of unknown etiology. Elevated serum tryptase levels in such cases could be indicative of the presence of a myeloid neoplasm. Thus, patients with a persistently elevated tryptase level should undergo a bone marrow examination in order to document or exclude the presence of a myeloid neoplasm unless HAT has been documented. In other cases, the KIT D816V test is performed before a bone marrow examination is recommended.<sup>72</sup> Finally, it is also important to know that a tryptase level within the normal range does not rule out the presence of a myeloid neoplasm.

#### **Conflict of Interest Declaration**

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