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The serum tryptase test: an emerging robust biomarker in clinical hematology

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Summary

During the past few years a number of molecular markers have been developed in clinical hematology, most of them related to specific gene defects. However, there is also an unmet need to develop novel serologic parameters to improve diagnostics and prognostication in daily practice. Among these, the serum tryptase appears to be a most reliable biomarker of myeloid neoplasms. Elevated tryptase levels are found in subgroups of patients with mastocytosis, myelodysplastic syndrome, myeloproliferative neoplasm, acute myeloid leukemia, chronic myeloid leukemia (CML) and chronic eosinophilic leukemia (CEL). In these patients, the tryptase level is of diagnostic and/or prognostic significance. In mastocytosis, an elevated tryptase level is a minor criterion of systemic disease and in BCR-ABL1⁺ CML, elevated tryptase at diagnosis correlates with treatment responses and overall survival. In patients with elevated tryptase, the enzyme also serves as follow-up parameter and can be employed to measure treatment-responses. In the current article, we review and update the perspectives of tryptase and provide recommendations for use of this conventional biomarker in daily practice.

Keywords

Tryptase; Biomarker; Prognostication; Myeloid Leukemias; Mastocytosis

Introduction

Molecular research has revolutionized clinical hematology in the past 15 years, mainly by introducing a plethora of molecular markers and promising targets in various hematologic malignancies. Highlighting examples are the *BCR/ABL1* fusion gene in chronic myeloid leukemia (CML) [1-3], mutations in *JAK2*, *CALR* and *MPL* in BCR/ABL1-negative myeloproliferative neoplasms (MPN) [4-6], *KIT* mutations in systemic mastocytosis (SM) [7-9] and *PDGFRA/B*- or *FGFR* mutations in myeloid neoplasms presenting with eosinophilia (MPN-eo) [10-12]. Other mutations have been identified in the complex field of myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML) [13-18]. In many instances, the abnormal gene products serve as disease-related criteria and/or targets of therapy. Although several markers are disease-specific, more and more data suggest that these mutations can also be expressed simultaneously in neoplastic cells, and that complex aberration profiles and signalling networks contribute to disease evolution, expansion of neoplastic cells and drug resistance [19-22]. In some of these patients, more than one driver mutation is expressed in neoplastic cells. In addition, epigenetic and stem cell-related factors as well as the disease-related microenvironment play a role in disease evolution and progression. On the other hand, more and more data suggest that a driver lesion *per se* is not necessarily sufficient to induce an overt malignancy. Rather, in most instances, these mutants are already expressed in small-sized clones in early phases of oncogenesis, often in apparently healthy individuals, and only later, after acquisition of additional pro-oncogenic lesions during disease evolution from a premalignant to a malignant state, these lesions become important drivers of oncogenesis and subclone formation [23-27]. Therefore, there is also a clear need for additional parameters and markers defining prognostic subgroups of patients in these malignancies, independent of molecular markers and aberration profiles.

During the past 10 years only a few robust serologic parameters have been established in clinical hematology. Out of these, the most important novel serologic parameter is the serum tryptase test [28-30]. In the current article, we review the clinical implications and value as well as limitations of this parameter in daily practice.

Cellular origin, historical overview and development of the test

Tryptase is a serine protease that is primarily produced and stored in mast cells (MCs) and less abundantly in blood basophils [31-34]. In tissue MCs, tryptase is produced and released in a constitutive manner, regardless of the organ, location of MCs, maturation stage or subtype of MCs [32]. Mature tissue MCs also store larger quantities of the enzyme in their metachromatic granules. Two major forms of tryptase are produced in MCs, alpha-tryptase and beta-tryptase [34-36]. Whereas the alpha form is produced and released constantly in MCs, the beta-form is primarily stored in MC granules [37]. During an anaphylactic reaction, MCs rapidly release substantial amounts of alpha- and beta tryptases. In the recovery period after degranulation, MCs also produce tryptase and re-granulate to 'fully equippe' tryptase+ effector cells [38].

Mature blood basophils store small quantities of tryptase in their granules and may release the enzyme during degranulation [34,39]. However, basophils do not synthesize or release larger quantities of tryptase and these cells also do not re-granulate after degranulation. An important aspect is that immature basophils in the bone marrow and leukemic basophils can produce and release abundant quantities of the enzyme [40].

The basal serum tryptase level in healthy individuals results from the constant release of the enzyme from mature tissue MCs [41]. During a severe anaphylactic reaction, the serum tryptase level may rapidly increase above baseline which is a diagnostic parameter of MC activation [42,43]. After such an event it may take several hours to days until tryptase levels return back to baseline. Therefore, the basal serum tryptase level has to be measured exclusively in the symptom-free interval when no signs or symptoms of anaphylaxis are seen which is of special importance in patients with mastocytosis or mast cell activation syndromes (MCAS). Otherwise, the serum tryptase test is simple, reliable and highly reproducible. There are no pre-analytical caveats and the serum samples can be shipped or stored before measurements.

During the past two decades, several attempts have been made to establish a robust assay for the measurement of tryptase. In an initial phase, an immunometric assay for the quantification of beta tryptase has been developed [44]. However, this assay is only useful for determining tryptase levels in patients with severe anaphylaxis. Later, a fluoroenzyme assay detecting all major (alpha and beta) tryptase species, including all pre-forms of the enzyme, has been developed [45]. This assay is now widely used and is commercially available.

Increased levels of basal serum (total) tryptase are found in patients with SM and in patients with other myeloid neoplasms [28,29,41]. The initial evaluation of tryptase as a diagnostic marker in hematology focused on mast cell disorders [41]. Notably, tryptase levels are almost always elevated in patients with SM, but are normal in most cases with cutaneous mastocytosis (CM) [41,46]. Therefore, a markedly elevated tryptase (>20 ng/ml) was introduced as a minor diagnostic criterion of SM by the World Health Organization (WHO) [47-49].

During the past few years we and others have shown that immature blast cells in myeloid leukemias also produce and express tryptase [50-52]. In addition, leukemic (immature) basophils, as opposed to normal blood basophils, express substantial amounts of the enzyme [40] (Table 1). Correspondingly, serum tryptase levels were found to be elevated in patients with diverse myeloid leukemias, including AML, CML and chronic eosinophilic leukemia (CEL) [28,29,50,52]. Interestingly, in each of these leukemias, elevated tryptase levels are detectable in distinct (prognostic) subgroups of patients.

Total serum tryptase levels in healthy individuals

The median serum tryptase level in healthy adults averages at about 5 ng/ml, with a range of <1 to 30 ng/ml. In more than 99% of healthy individuals, the serum tryptase level is below or equal to 15 ng/ml [28,45,53]. Nevertheless, many centers consider the normal range as being <11.4 ng/ml (cut off provided by company) or even <10 ng/ml. In this regard, it has to

be mentioned that the serum tryptase level in healthy individuals increases with age [28,54] and that the original assay showed false-positive results due to heterophilic antibody-interference [54-56]. As a result, initial studies had included control-cohorts where tryptase levels were relatively low. Whatever the normal cut off is considered to be in various centers, there are clearly many healthy individuals who have a serum tryptase level between 10 and 15 ng/ml, and there are even healthy individuals in whom the serum tryptase level exceeds 20 or even 25 ng/ml [28,45].

There are also subtle differences in median serum tryptase levels between women and men [28]. However, the basal serum tryptase level remains <15 ng/ml in most (>95%) healthy individuals, regardless of age or sex. In addition, tryptase levels remain remarkably stable during viral or bacterial infections or other inflammatory disease processes [28]. However, tryptase levels may slightly increase during a chronic active helminth infection and in patients with renal failure [28,57,58]. Finally, as mentioned, tryptase levels may transiently increase during an anaphylactic reaction [42-44].

Elevated basal serum tryptase levels are frequently detected in patient with myeloid neoplasms

Elevated serum tryptase levels (>15 ng/ml) are detectable in patients with SM, MDS, MPN, AML, CML and CEL [28,29,41,50,52]. The percentage of cases with elevated tryptase varies depending on the type of disease. Almost all patients with SM (>90%) present with an elevated serum tryptase. In addition, elevated serum tryptase levels are found in about 30-40% of all patients with AML [50,52] and in about 30-35% of all patients with CML [28]. The incidence is lower in patients with MPN and MDS [28,53]. However, in those patients with MPN or CEL in whom a *FIP1L1/PDGFR*A fusion gene is present, elevated tryptase levels are frequently detected [28,59,60]. In patients with lymphoid neoplasms, including lymphomas and multiple myeloma, tryptase levels are usually within normal range [28]. Moreover, tryptase levels are normal in patients with non-hematologic malignancies [61-63].

Value of tryptase testing in patients with mastocytosis

In patients with suspected SM, the tryptase level is widely used as a robust screen-parameter [43,46,64-67]. In those with clearly elevated tryptase (>20 ng/ml) the likelihood of SM is rather high, especially in adults with typical skin lesions and in those who are suffering from a known hymenoptera venom allergy [43,46,63-67]. In a vast majority of these patients, bone marrow investigations reveal SM and the *KIT* mutation D816V is detectable [63-67]. Therefore, a clearly elevated serum tryptase level serves as a minor SM criterion in the WHO classification [47-49]. By contrast, in patients with cutaneous mastocytosis (CM), serum tryptase levels are usually <20 ng/ml or even within normal range [43,46,47,68]. In SM, the serum tryptase concentrations vary among patients, depending on the subtype of SM and burden of neoplastic MCs [47,68]. In patients with advanced SM, including aggressive SM (ASM) and mast cell leukemia (MCL), serum tryptase levels are usually very high (>200 ng/ml) (Table 2) [68]. In patients with indolent SM (ISM), tryptase levels may be low or high, depending on the total mass of MCs [28,47,68]. In those with very high

serum tryptase levels, the smouldering subtype of SM (SSM) may be diagnosed (Table 2) [68-70]. In fact, a tryptase level >200 ng/ml serves as a criterion of SSM [47,69]. However, in contrast to ASM and MCL, tryptase levels in patients with ISM and SSM usually remain in a stable range, without tendency to increase over time [28]. By contrast, in patients who have or progress to MCL, serum tryptase levels often increase, sometimes up to >1000 ng/ml.

Serum tryptase levels in AML and implications of elevated tryptase for diagnostics and prognostication

A number of previous and more recent data suggest that blast cells express and release MC tryptase in a substantial number of patients (30-40%) with AML [28,50,52]. It has also been described that tryptase levels at diagnosis correlate with the subtype of AML as well as with the karyotype in these patients [50]. The highest median serum tryptase levels are measured in patients with inv(16), t(15;17) or t(8;21) (Table 2) [50]. In a subset of patients with AML in whom an elevated tryptase level is recorded, MC-lineage involvement and the *KIT* D816V mutation are found. In these cases, overt SM (SM-AML) is usually diagnosed [71-73]. However, in most AML patients who present with elevated serum tryptase (>70%), the final diagnosis is 'tryptase+ AM' without concomitant SM. In patients with elevated tryptase at diagnosis, tryptase levels can be monitored and employed to measure minimal residual disease [52]. In those in whom a persistently elevated tryptase level is found at the time of first complete remission (CR) or increase again at a later time point, the prognosis is poor and relapses are seen in over 90% of cases [52]. In other words, an elevated serum tryptase level in AML after successful chemotherapy is associated with a reduced relapse-free survival and thus reflects a poor prognostic factor. There is one important exception to this rule which is SM-AML [74]. In these patients, serum tryptase levels do not decrease substantially during and after chemotherapy (or stem cell transplantation) because the SM component of the disease is usually resistant. However, this does not mean that the AML fraction of the clone is also resistant. Rather, there are patients with SM-AML who were transplanted and achieved CR from AML and long-term relapse-free survival even if their SM-component of the disease persisted.

Prognostic value of serum tryptase levels in Ph+ CML

In BCR/ABL1⁺ CML, tryptase is primarily produced by immature basophils and basophil-committed (non-granulated) progenitor cells [40]. In line with this notion, serum tryptase levels are almost always elevated in the accelerated phase (AP) of the disease where basophilia is a characteristic (diagnostic) feature (Table 2) [28,75]. Moreover, serum tryptase levels correlate with basophil counts. In patients with chronic phase (CP) CML, serum tryptase levels are either normal or elevated (>15 ng/ml) [28,75,76]. Most CP patients with elevated tryptase have basophilia and are within the high risk group of the European Treatment Outcome Study (EUTOS) score or Sokal score [76]. Moreover, CP patients with elevated tryptase (>15 ng/ml) have a high risk of progression and a shorter survival compared to patients with normal serum tryptase levels [75,76]. Based on these observations, the serum tryptase has value as a prognostic biomarker in newly diagnosed CML. Whether serum tryptase levels can replace basophil counts in currently available

prognostic scoring systems used to classify patients with freshly diagnosed CML, such as the EUTOS score, is currently under investigation [76]. Indeed, that implementation of tryptase (replacing basophil counts) in the EUTOS score may enhance the prognostic value of this score with regard to survival-prediction in CML patients treated with imatinib [76].

Serum tryptase levels in other myeloid neoplasms: MDS, MPN and CEL

Serum tryptase levels are also elevated in distinct subsets of patients suffering from MDS, MPN and CEL [28]. One defined subset are patients exhibiting a *FIP1L1/PDGFR* fusion gene product in neoplastic cells (Table 2) [28,59,60]. In these patients, the underlying disease may be CEL, MPN or a MDS/MPN overlap disease presenting with marked eosinophilia (MPN-eo or MPN/MDS-eo). In these patients, clonal CD25+ mast cells may be detectable and are considered to contribute to the elevated tryptase level. In a few of these patients, an associated SM (SM-CEL) may be detected [77,78]. However, there are also patients with MPN or MDS with elevated tryptase, in whom no *PDGFR* fusion gene is detectable [28,53]. Some of these patients have MC hyperplasia or an increase in immature basophils [28,53]. In other patients, an increase in tryptase+ blast cells is demonstrable [53]. However, in contrast to AML and CML, tryptase levels have no major diagnostic or prognostic impact in MDS or MPN [28,53]. Therefore, we do not recommend the use of tryptase in patients with known MDS and MPN with a few exceptions: first, in patients with MDS or MPN in whom an accompanying basophilia or eosinophilia is recorded, a serum tryptase test should be performed. Some of these patients may suffer from a *PDGFR*- or *FGFR*-mutated type of disease or from concomitant SM. Finally, in a few patients, serum tryptase levels are very high at diagnosis or increase during disease progression, so that tryptase levels can be employed as follow-up parameter. All in all, we recommend to apply the tryptase test only once at diagnosis in most patients with MDS or MPN. However, in distinct cases, serial determinations of tryptase may be useful in daily practice.

Tryptase is a recommended screen parameter in clinical hematology

Based on the selective increase in basal tryptase levels in various groups of patients with myeloid neoplasms and the obvious diagnostic and prognostic potential of this parameter, the serum tryptase test has been established as a screen-marker in clinical hematology in many centers, especially those where SM is also diagnosed. In this regard it should be pointed out again that the serum tryptase assay is a very robust and reliable test-system. Tryptase levels should be measured as a screen-parameter in patients with suspected (systemic) mastocytosis, in patients with (suspected) CEL and in patients with other myeloid neoplasm where involvement of the mast cell-, basophil- or eosinophil lineage is suspected (Table 3). In acute leukemias, an elevated tryptase may help to differentiate between myeloid (AML) or lymphoblastic (ALL) leukemia, and in patients with marked eosinophilia, an elevated serum tryptase level may reveal a CEL or an underlying SM. In patients with CML, an elevated tryptase may be indicative of the presence of an (occult) accelerated phase which is prognostic and may influence treatment decisions. In patients with SM, the tryptase level correlates with the burden of neoplastic MC and with the subtype of SM [28,68]. A normal tryptase level may confirm the presence of cutaneous mastocytosis as most patients with SM have an elevated tryptase level. It is noteworthy that elevated tryptase levels may

also be detected in familial MC activation syndromes with or without a concomitant SM [79]. Finally, serum tryptase may be used as a non-invasive screen-parameter in all patients with unexplained cytopenias or unexplained leukocytosis or thrombocytosis (Table 3). In such patients, an elevated tryptase level increases the likelihood the patient is suffering from a myeloid neoplasm, whereas patients with reactive blood count abnormalities, e.g. infections or autoimmune disorders, but also solid tumors, usually have a normal serum tryptase level. However, it should be pointed out again here, that a normal serum tryptase level does not exclude the presence of a myeloid neoplasm. In those with a persistently elevated tryptase level, a bone marrow examination is always required in order to document or exclude the presence of a myeloid neoplasm.

Concluding remarks and future perspectives

During the past 5 years, the serum tryptase test has been implemented as a robust biomarker of myeloid malignancies. In SM, a clearly elevated serum tryptase level (>20 ng/ml) serves as a minor SM criterion. In CEL and MPN-eo with mutated *PDGFRs*, an elevated serum tryptase level is also a useful screening parameter. Other myeloid neoplasms typically presenting with elevated tryptase are high-risk BCR/ABL1⁺ CML and AML with inv(16), t(15;17) or t(8;21). In each disease subset with elevated tryptase, enzyme levels can be measured in the follow up and employed for monitoring disease activity, treatment responses and the residual disease burden after cytoreduction. In CML and AML, tryptase levels are also of prognostic significance. It can be expected that serum tryptase test will gain further acceptance as a global standard-marker of myeloid neoplasms in clinical hematology.

Expert Commentary

The tryptase test should be applied as a standard serologic parameter of myeloid neoplasms in clinical hematology in daily practice. Tryptase measurements are recommended as a broad screening-test in patients with suspected myeloid neoplasms, including those who have unexplained cytopenias, unexplained leukocytosis, unexplained thrombocytosis, unexplained eosinophilia, unexplained basophilia or other clinical signs or symptoms indicative for the presence of a myeloid neoplasm, including mastocytosis. In such patients, an elevated tryptase increases the likelihood of an underlying myeloid neoplasm and is thus in favour of a bone marrow examination. In addition, tryptase is recommended as a diagnostic and prognostic parameter in patients with known mastocytosis, CEL, CML and AML. Furthermore, tryptase levels should be measured in the follow up and used as treatment response parameter for those patients in whom the tryptase level was elevated at diagnosis.

Five-Year View

Within the next 5 years, the serum tryptase test will be further implemented as a robust and important standard and screen parameter in clinical hematology. Our expectation is that more and more hematology centers will employ the tryptase test in order to screen for myeloid malignancies, to exclude or document systemic mastocytosis, to subtype and grade myeloid neoplasms, and to improve prognostication in certain leukemias, such as CML. The

impact of tryptase in myeloid neoplasms will be at least the same as that of $\beta 2$ -microglobulin in the lymphoid malignancies.

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Key Issues

1. The serum tryptase test is a novel reliable biomarker for various myeloid neoplasms
2. The serum tryptase test can be used as screening parameter in patients with suspected myeloid neoplasms, including systemic mastocytosis and eosinophilic leukemias
3. In patients with mastocytosis, an elevated tryptase level is indicative of systemic involvement and helpful to grade and sub-classify the disease
4. In BCR/ABL1⁺ CML, an elevated tryptase at diagnosis correlates with basophilia, treatment responses and with the overall survival and thus prognosis
5. In patients with CEL or MPN-eo, an elevated serum tryptase level correlated with the presence of a *FIP1L1-PDGFR*A fusion gene
6. In patients with AML, markedly elevated levels of serum tryptase are found in various groups of patients, including those in whom blast cells express *PML-RARA*, *AML1-ETO* or *CBFB-MYH11*
7. In AML patients who are in complete hematologic remission after chemotherapy a persistently elevated tryptase level is associated with an increased risk of relapse
8. In patients with markedly elevated tryptase at diagnosis, the serum tryptase level should be measured in the follow up; a further increase is often associated with disease progression; and in those who receive interventional treatment, the serum tryptase test can be employed to document and measure treatment responses

Table 1

Cellular source of elevated serum tryptase in myeloid neoplasms

Neoplasm	source of elevated tryptase
Systemic mastocytosis (SM)	neoplastic mast cells
Mast cell leukemia (MCL)	neoplastic/leukemic mast cells
Chronic eosinophilic leukemia	neoplastic mast cells
<i>FIP1L1-PDGFR</i> A + neoplasm	neoplastic mast cells
Acute myeloid leukemia	myeloblasts
Chronic myeloid leukemia	neoplastic immature basophils
Myelodysplastic syndromes (MDS)	basophils, mast cells or blasts
Myeloproliferative neoplasms (MPN)	basophils, mast cells or blasts
MDS/MPN overlap syndromes	basophils, mast cells or blasts
Myelomastocytic leukemia (MML)	leukemic mast cells

Table 2

Myeloid neoplasms with markedly elevated serum tryptase*

Myeloid neoplasm	subset of patients with particularly elevated serum tryptase
Systemic mastocytosis (SM)	Smoldering SM (SSM) Aggressive SM (ASM) Mast cell leukemia (MCL) SM-AHNMD
Chronic eosinophilic leukemia	<i>FIP1L1/PDGFR</i> A+ subset
Acute myeloid leukemia (AML)	AML with inv(16) [<i>CBFB-MYH11</i>] AML with t(15;17) [<i>PML-RARA</i>] AML with t(8;21) [<i>AML1-ETO</i>]
Chronic myeloid leukemia (CML)	Accelerated phase (AP)
Myelodysplastic syndromes (MDS)	-
Myeloproliferative neoplasms (MPN)	-
MDS/MPN overlap syndromes	CMML subset
Myelomastocytic leukemia (MML)	-

SM-AHNMD, SM with associated clonal hematologic non-mast cell-lineage disease; CMML, chronic myelomonocytic leukemia.

* Markedly elevated tryptase: >20 ng/ml.

Table 3**Indications for a tryptase test in clinical hematology**Pre-diagnostic (screen):

Suspected systemic mastocytosis (SM)
 Mastocytosis in the skin (CM versus SM)
 Suspected myeloid neoplasm other than SM
 Unexplained cytopenia
 Unexplained leukocytosis
 Unexplained thrombocytosis
 Suspected mast cell activation syndrome (MCAS)

At diagnosis:

Acute myeloid leukemia (AML)
 Chronic myeloid leukemia (CML)
 Chronic eosinophilic leukemia (CEL)
 Myelodysplastic syndromes with hypereosinophilia (MDS-eo)
 Myeloproliferative neoplasms with hypereosinophilia (MPN-eo)
 Neoplasms with mutated *PDGFRA*
 Neoplasms with mutated *PDGFRB*
 Neoplasms with mutated *FGFR*
 Mastocytosis: CM and SM, including SM-AHNMD

During follow-up:

CM and SM, including SM-AHNMD
 AML with elevated tryptase (T+ AML)
 CEL with elevated tryptase
 Neoplasms with mutated *PDGFR* or *FGFR* with elevated tryptase
 MDS-eo and MPN-eo with elevated tryptase

Suspected mast cell activation syndrome (MCAS)*:

Unexplained hypotension
 Unexplained histamine/mediator-related symptoms
 Unexplained pruritus, flushing or exanthema

CM, cutaneous mastocytosis; AHNMD, associated hematologic non-mast cell-lineage disease; PDGFR, platelet derived growth factor receptor; FGFR, fibroblast growth factor receptor.

* In these patients tryptase levels are compared to baseline levels if possible. A clear elevation from baseline (20% plus total 2 ng/mL) is a criterion of MCAS [30].