

ImmunoCAP® Tryptase
Clinical utility of Total Tryptase

Background

Tryptase is the most abundant protein in mast cells. It is also found in basophils but in much smaller amount.

ImmunoCAP® Tryptase measures the total tryptase of all inactive proforms of α -tryptase and β -tryptase, as well as the enzymatically active mature β -tryptase.

Tryptase proforms are continuously released from the mast cells into the bloodstream reflecting the number of mast cells.

They constitute the baseline levels in healthy individuals.

Elevated baseline tryptase levels serve as a risk marker for certain patients to get into severe anaphylactic reactions, especially after parenteral introduction of substances as by insect venoms and drugs

Pathological increased levels of tryptase proforms reflect the mast cell burden in certain haematological abnormalities and neoplasms, and the severity grade in systemic mastocytosis.

Mature tryptase is stored in the granula of resting mast cells. It is released into the bloodstream during mast cell activation, either by IgE- or non-IgE mediated mechanisms. The transiently increased levels of mature tryptase serve as a clinical marker confirming severe reactions as anaphylaxis.

References: 1-4, 18.

Tryptase – a mast cell marker

ImmunoCAP Tryptase measures total tryptase = sum of proforms and mature tryptase

Tryptase proforms

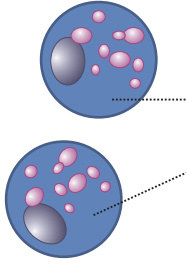
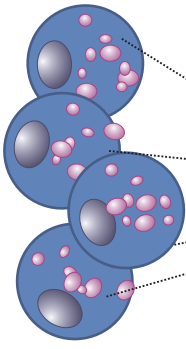
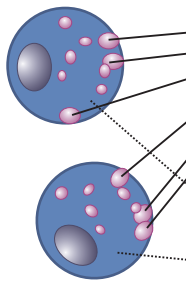
Risk marker for severe reactions

Diagnosis and therapy follow-up of Haematological neoplasms and Mastocytosis

Mature tryptase

Confirmation of Anaphylaxis

Clinical utility of Total Tryptase

Mast cells	Total Tryptase in health and disease		Tryptase levels
<p>Resting</p> 	<p><u>Proforms</u> α-tryptase β-tryptase</p>	<p>Baseline levels in healthy individuals</p>	<p>1 ~ 15 µg/l</p>
Risk patients	<p>Elevated baseline levels Underlying mastocytosis</p>		<p>> 10 µg/l</p>
<p>Increased levels</p> 	<p><u>Proforms</u> α-tryptase β-tryptase</p>	<p>Systemic mastocytosis Haematological neoplasms</p>	<p>> 20 µg/l >> 200 µg/l</p>
<p>Activated</p> 	<p><u>Mature</u> β-tryptase <u>Proforms</u> α-tryptase β-tryptase</p>	<p>Anaphylactic reaction Severe or fatal systemic reactions</p>	<p>Transiently elevated from baseline</p>

ImmunoCAP Tryptase measures Total Tryptase

Individual tryptase baseline levels

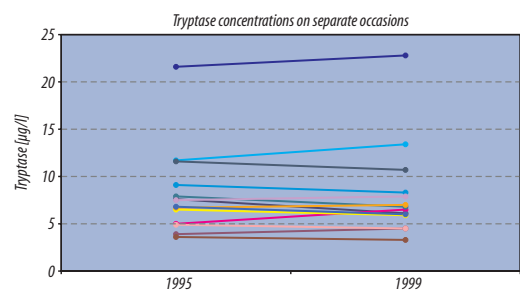
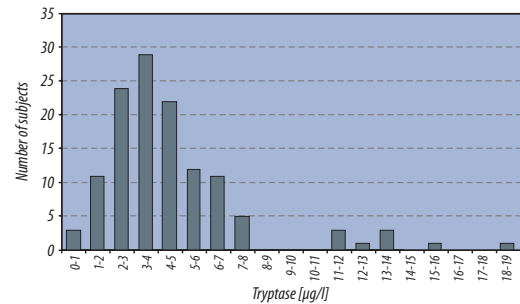
In healthy individuals the baseline levels have been reported to range approximately between 1-15 µg/l.

In an internal study performed at Phadia of 126 apparently healthy individuals, the range was 2-12 µg/l considering two standard deviations from the mean. However, healthy individuals may have baseline levels up to approximately 20 µg/l.

Each individual has its own unique baseline level, which usually is stable over time.

In a study including apparently healthy individuals, the baseline tryptase level was almost unchanged in samples collected 4 years apart. This study was performed at Phadia.

References: 5, 6, 20, 21.



Risk marker for severe reactions

Some individuals with elevated baseline levels of tryptase, approximately > 10 µg/l, are considered to be at increased risk for severe anaphylactic reaction. Levels between approximately 10 to 20 µg/l reflect an increased mast cell burden even without necessarily being an underlying mastocytosis. The increased risk is more predominant in individuals with a known history of severe systemic reactions.

Elevated tryptase levels as a risk factor have especially been evaluated in the investigation and treatment of severe reactions to insect venoms (SIT).

Elevated baseline levels may also serve as a risk factor for severe reactions during surgery where the reactions are caused by anaesthetics and other perioperative substances.

References: 1, 10, 12-14, 22.

**Elevated baseline tryptase levels,
a risk factor for severe reactions**

Risk patients with severe reactions to insect venoms and drugs

Tryptase as a clinical marker in severe insect venom reactions

Venoms especially from hymenoptera, commonly bees, wasps and ants, may cause the onset of severe anaphylactic reactions.

In average the prevalence of systemic anaphylactic sting reactions is around 3%, varying between 0.3% and 7.5% in self reported studies.

Up to 25% of patients with severe reactions have an elevated baseline tryptase level.

The importance of identifying patients with elevated baseline levels has been emphasized as they are predisposed for severe anaphylactic reactions.

Increased levels may be, but need not necessarily be, due to an underlying mastocytosis.

Guidelines have been established in EAACI Positions Papers for

- ◆ Diagnosis
- ◆ Prevention and Treatment

The clinical utility of measuring transiently increased tryptase levels has thoroughly been evaluated in the diagnosis and treatment of patients suffering severe insect reactions.

When severe reactions occur, blood samples should be taken during the reaction to verify a transient increase of tryptase, confirming activation of mast cells.

The mechanism may either be IgE-mediated or non-immunological, directly triggered by the venom components.

Specific IgE antibody testing aids in the diagnosis of venom allergy.

Elevated baseline tryptase levels suggest special patient care

- ◆ during immunotherapy (SIT)
 - special caution on each treatment occasion
- ◆ post-SIT
 - lifelong treatment considered
 - EpiPen always available

References: 12-14, 22.

Prevalence

Identify risk patients with elevated baseline tryptase levels

EAACI Positions Papers 2005

Confirmation of Anaphylaxis

Triggering mechanism

Immunotherapy management

Tryptase as a clinical marker in perioperative severe reactions

Severe reactions during anaesthesia and surgery are rare. However, when occurring it is important to identify the mechanism and underlying cause. The first signs recognized by the surgeons and anaesthetists are commonly the severe symptoms of bronchospasm and cardiovascular collapse causing the emergent situation.

Elevated baseline tryptase levels indicate an increased mast cell burden and may serve as a risk factor for severe reactions during surgery.

Sensitisation, i.e. presence of Specific IgE antibodies to substances which the patients are exposed to during surgery is another risk factor.

To confirm an anaphylactic reaction, the importance of measuring the transient increase of tryptase during the perioperative phase is well established.

During the perioperative phase the patients are exposed to multiple agents possible to cause severe potentially life-threatening adverse reactions.

Examples of triggering agents:

- ◆ Neuromuscular blocking relaxants - NMBA
- ◆ Natural Rubber Latex
- ◆ Antibiotics
- ◆ Disinfectants
- ◆ Hypnotics
- ◆ Colloids
- ◆ Opioids
- ◆ Other substances

References: 8-10.

Risk marker in surgery

Case history important

Confirmation of Anaphylaxis

Triggering factors

Succinylcholine

Latex (gloves, catheters)

Penicillin (β -lactams)

Chlorhexidine

Thiopental

Dextran

Morphine

Radiocontrast media

Time course of elevated mature tryptase

The **time course** of transiently elevated mature tryptase must be considered for the verification of a mast cell activation.

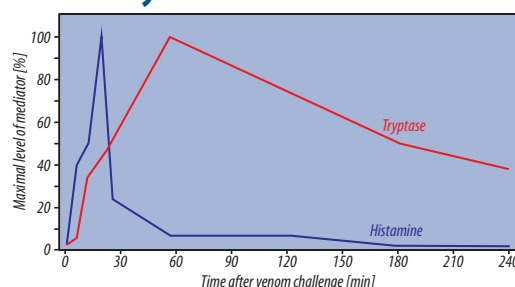
The peak level is usually reached 15-120 minutes after onset of the reaction, and then the tryptase level declines slowly within the next 3-6 hours. The biological half-life for tryptase is about two hours.

The return to baseline level can generally be verified approximately 24 hours after the reaction. If levels still are above normal range another sample should be taken after 1-2 weeks to establish baseline levels.

References: 1, 8-11.

The elimination profile is best verified collecting three consecutive samples

- **1st sample taken 15 min-3 hours after onset of the symptoms**
- **2nd sample taken between 3-6 hours**
- **3rd sample taken 24-48 h to verify the return to baseline**



Examples of elimination profiles for verification of the return to baseline levels

1. "Normal" elimination profiles. Patient case adapted from T. Dybendal *et al.* "Screening for mast cell tryptase and serum IgE antibodies in 18 patients with anaphylactic shock during general anaesthesia".

Acta Anaesthesiol Scand 2003;47:1211-1218 (8).

2. Elimination profile totally within the normal range. Patient case adapted from Y. Nakaigawa *et al.* An anaphylactic reaction to blood supplied from patient's mother.

Acta Anaesthesiol Scand 2002;46:1276-1278 (25).

3. Elimination profile with baseline outside normal range – Risk patient with suspected underlying mastocytosis identified. Patient case adapted from Alonso Diaz de Durana *et al.* Anaphylaxis during negative penicillin skin prick testing confirmed by elevated serum tryptase.

Allergy 2003;58:159. (26).

Time	Tryptase profile µg/l
0.17 h	57
3 h	38
6 h	18
24 h	5

Time	Tryptase profile µg/l
0.5 h	10
1 h	9.5
3 h	10,9
6 h	10
24 h	2.1

Time	Tryptase profile µg/l
30 min	180
90 min	69
"next few hours"	"decreased further"
1 month	17.3
1 – 18 months	12.8 – 17.3

When to expect transient increased levels of tryptase

Transient increases of tryptase are most commonly measurable in the blood after severe anaphylactic reactions, causing respiratory and cardiovascular symptoms. However, also in less severe reactions transient increases can be measured in blood.

Changes in tryptase levels can also be measured locally after allergen provocations, e.g. in nasal secretions.

Both the occurrence and the magnitude of increased tryptase are more pronounced after IgE mediated reactions than after non-immunological reactions, as well as more commonly seen after parenteral than oral or inhaled introduction of mast cell activation substances.

Post mortem measurement of increased levels of mature β -tryptase can be an additional diagnostic tool when an anaphylactic reaction is suspected as cause of death .

References: 1, 10, 15, 16, 17, 23.

Anaphylactic reactions

Severity of the reactions

IgE-mediated

Parenteral introduction

Post mortem

Tryptase in mastocytosis

Persistently elevated levels of tryptase proforms in blood reflect the increased abnormal mast cell burden in mastocytosis. These heterogeneous diseases are commonly associated with a mutation in the receptor for the stem cell factor (SCF), a cytokine enhancing survival and autonomous growth of the mast cells.

Only the skin is pathologically infiltrated with mast cells in **cutaneous mastocytosis (CM)**, as in urticaria pigmentosa.

In general tryptase levels is $<20 \mu\text{g/l}$.

In systemic mastocytosis (SM) skin lesions is the most common symptom and in addition the bone marrow and/or other extracutaneous organ(s) such as the liver, spleen, gastrointestinal tracts and the lymph nodes are affected.

In most cases the tryptase levels is $>20 \mu\text{g/l}$.

Systemic mastocytosis is subdivided into categories with different severity grades

- ◆ Indolent systemic mastocytosis (ISM)
- ◆ Bone marrow mastocytosis (BMM)
 - a subgroup of ISM isolated to the bone marrow
- ◆ Smoldering systemic mastocytosis (SSM)
- ◆ Systemic mastocytosis (SM) associated with a haematologic clonal non-mast cell lineage disorder (SM-AHNMD)
- ◆ Aggressive systemic mastocytosis (ASM)

Malignant forms of mastocytosis are

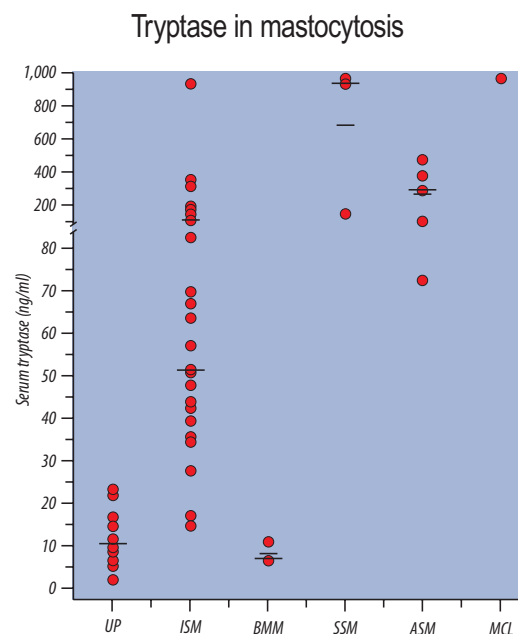
- ◆ Mast cell leukemia (MCL)
- ◆ Mast cell sarcoma

The tryptase levels are overlapping between different categories of the disease. When SSM is established the tryptase levels are commonly $>200 \mu\text{g/l}$ and in the more aggressive forms $\gg 200 \mu\text{g/l}$.

Systemic mastocytosis is a risk factor for anaphylactic reactions particular in response to insect sting and drugs.

References: 1, 5, 6, 10, 19, 24.

Total tryptase determinations are recommended by the World Health Organization (WHO) as a minor diagnostic criterion of suspected systemic mastocytosis



Sperr WR, Jordan JH, Fiegle M et al: *Int Arch Allergy Immunol* 2002;128:136-141.

With permission from S. Karger AG, Basel.

Tryptase levels correspond to severity

Increased risk for anaphylactic reactions

Tryptase in haematological neoplasms

Diagnostic and prognostic significance of tryptase is seen in haematological abnormalities and malignancies (neoplasms) irrespective if systemic mastocytosis is established or not.

These haematological disorders involve uncontrolled growth of immature myeloid cells in the bone marrow and/or the circulation.

In addition to mutations as in mastocytosis, other chromosomal and gene defects are also common causes to the development of these diseases.

- ◆ Acute myeloid leukemia (AML)
- ◆ Chronic myeloid leukemia (CML)
- ◆ Myeloproliferative disorders (MPD)
- ◆ Myelodysplastic Syndrom (MDS)
- ◆ Chronic myelomonocytic leukemia (CMML)
- ◆ Other myeloid neoplasm
- ◆ Chronic eosinophilic leukemia (CEL)

Haematological non-mast cell disorders, e.g. Chronic eosinophilic leukemia (CEL) associated with a systemic mastocytosis (SM) are designated as SM-AHNMD.

References: 1, 5.

Non-mast cell disorders associated with elevated tryptase levels in haematological neoplasms

Patients (%) with elevated tryptase levels

- ***AML 30-40%***
- ***CML 40-50%***
- ***MPD without CML 5-40%***
- ***MDS 20-30%***
- ***CMML 40-60%***

SM-AHNMD – a specific category of SM in the World Health Organization (WHO) classification of mastocytosis

Tryptase – a monitoring and prognostic marker

Several effective targeting drugs have been developed for cytoreductive therapy of systemic mastocytosis and haematological neoplasms. During treatment tryptase measurements is a useful monitoring and prognostic tool.

References: 1, 5, 7.

Mast cell reductive therapy

References

1. Schwartz LB.
Diagnostic Value of Tryptase in Anaphylaxis and Mastocytosis.
Immunol Allergy Clin N Am 2006;26:451-463.
2. Schwartz LB.
Mast Cells and Basophils.
Clin Allergy Immunol 2002;16:3-42.
3. Caughey GH.
Tryptase genetics and anaphylaxis.
J Allergy Clin Immunol 2006;117(6):1411-1414.
4. Metcalf DD and Boyce JA.
Mast cell biology in evolution.
J Allergy Clin Immunol 2006;117(6):1227-1229.
5. Valent P.
Diagnostic Evaluation and Classification of Mastocytosis.
Immunol Allergy Clin N Am 2006;26:515-534.
6. Sperr WR, Jordan J-H, Fiegl M, Escribano L, Bellas C, Dirnhofer S, Semper H, Simonitsch-Klupp I, Horny H-P and Valent P.
Serum Tryptase Levels in Patients with Mastocytosis: Correlation with mast Cell Burden and Implication for Defining the Category of Disease.
Int Arch Allergy Immunol 2002;128:136-141.
7. Butterfield JH, Tefferi A, Kozuh GF.
Successful treatment of systemic mastocytosis with high-dose interferon-alfa: long-term follow-up of a case.
Leukemia Research 2005;29:131-134.
8. Dybendal T, Guttormsen AB, Elsayed S, Askeland B, Harboe T and Florvaag E.
Screening for mast cell tryptase and serum IgE antibodies in 18 patients with anaphylactic shock during general anaesthesia.
Acta Anaesthesiol Scand 2003;47:1211-1218.
9. Lieberman P, Kemp SF, Oppenheimer J *et al*.
The Diagnosis and Management of Anaphylaxis: An Updated Practice Parameter.
J Allergy Clin Immunol 2005;115:S483-523.
10. Ebo DG, Fisher MM, Hagendorens MM, Bridts CH, Stevens WJ.
Anaphylaxis during anaesthesia: diagnostic approach.
Allergy 2007;62:471-487.
11. Schwartz LB, Yunginger JW, Miller J, Bokhari R and Dull D.
Time Course of Appearance and Disappearance of Human Mast Cell Tryptase in the Circulation after Anaphylaxis.
J Clin Invest 1989;83:1551-1555.
12. Haerberli G, Brönnimann M, Hunziker T and Müller U.
Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy.
Clin Exp Allergy 2003;33:1216-1220.
13. Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JNG & the EAACI Interest Group on Insect Venom Hypersensitivity:
Diagnosis of Hymenoptera venom allergy.
Allergy 2005;60:1339-1349 / EAACI Position Paper <http://www.eaaci.net/media/PDF/D/652.pdf>.

14. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Müller U and the EAACI Interest Group on Insect Venom Hypersensitivity: Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice.
Allergy 2005;60:1459-1470 / EAACI Position Paper <http://www.eaaci.net/media/PDF/P/653.pdf>.
15. Yunginger JW, Nelson DR, Squillace DL, Jones RT, Holley KE, Hyma BA, Biedrzycki L, Sweeney KG, Sturmer WQ and Schwartz LB. Laboratory Investigation of Deaths Due to Anaphylaxis.
J Forensic Sci 1991;36:857-65.
16. Edston E, van Hage-Hamsten M.
Mast cell tryptase and hemolysis after trauma.
Forensic Science International 2003;131:8-13.
17. Jacobi HH, Skov PS, Poulsen LK, Malling H-J and Mygind N.
Histamine and tryptase in nasal lavage fluid after allergen challenge: Effect of 1 week of pre-treatment with intranasal azelastine or systemic cetirizine.
J Allergy Clin Immunol 1999;103(5):768-772.
18. Schwartz LB.
Mediators of human mast cell and human mast cell subsets.
Annals of Allergy 1987;58:226-37.
19. Valent P, Horny H-P, Escribano L, *et al*.
Diagnostic criteria and clasification of mastocytosis: a consensus proposal. Conference Report of "Year 2000 Working Conference on Mastocytosis".
Leuk Res 2001;25:603-25.
20. Schwartz LB, Sakai K, Bradford TR, *et al*.
The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis.
J Clin Invest 1995;96:2702-10.
21. Akin C, Metcalfe DD.
Surrogate markers of disease in mastocytosis.
Int Arch Allergy Immunol 2002;127:133-6.
22. Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M.
Basal Serum Tryptase Level Correlates With Severity of Hymenoptera Sting and Age.
J Investig Clin Immunol 2007;Vol.17(2):65-69.
23. Low I, Stables S.
Anaphylactic deaths in Auckland, New Zealand: a review of coronial autopsies from 1985 to 2005.
Pathology 2006(August);38(4):328-332.
24. Biedermann T, Ruëff F, Sander CA, Przybilla B.
Mastocytosis associated with severe wasp sting anaphylaxis detected by elevated serum cell tryptase levels.
British Journal of Dermatology 1999;141:1110-1112.
25. Y. Nakaigawa *et al*.
An anaphylactic reaction to blood supplied from patient's mother.
Acta Anaesthesol Scand 2002;46:1276-1278
26. Alonso Diaz de Durana *et al*.
Anaphylaxis during negative penicillin skin prick testing confirmed by elevated serum tryptase.
Allergy 2003;58:159.

Clinical utility of ImmunoCAP® Tryptase

Risk marker for severe reactions

- ◆ elevated baseline levels indicate increased risk for severe reactions (1-3)
 - in insect and drug allergy
 - before and during venom SIT (Specific ImmunoTherapy)

Anaphylactic reactions

- ◆ transient elevated levels
 - confirming mast cell activation
 - post mortem diagnosis

Marker for haematological neoplastic disorders and mastocytosis

- ◆ persistent elevated / increasing tryptase levels indicate haematological malignances
 - diagnosis and prognosis
 - follow up of therapy

Phadia

Phadia AB, P O Box 6460, SE-751 37 Uppsala, Sweden
Tel +46 18 16 50 00. www.phadia.com

Head office Sweden +46 18 16 50 00 Austria +43 1 270 20 20 Belgium +32 2 749 55 15 Brazil +55 11 3345 5050 Denmark +45 7023 3306 Finland +358 9 8520 2560 France +33 1 61 37 34 30 Germany +49 761 47 8050
Great Britain/Ireland +44 1 908 84 70 34 Italy +39 02 641 634 11 Japan +81 3 5365 8332 Netherlands +31 30 602 3700 Norway +47 21 67 32 80 Portugal +351 21 423 5350 Spain +34 935 765 800 Sweden +46 18 16 50 00
Switzerland +41 43 343 4050 Taiwan +886 2 2516 0925 United States +1 800 346 4364 Other countries +46 18 16 56 16