ImmunoCAP® Tryptase

Clinical utility of Total Tryptase

Phadia
**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.

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**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

<table>
<thead>
<tr>
<th>Mast cells</th>
<th>Total Tryptase in health and disease</th>
<th>Tryptase levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>Proforms &lt;br&gt;α-tryptase &lt;br&gt;β-tryptase</td>
<td>Baseline levels in healthy individuals</td>
</tr>
<tr>
<td>Risk patients</td>
<td>Elevated baseline levels &lt;br&gt;Underlying mastocytosis</td>
<td>&gt; 10 µg/l</td>
</tr>
<tr>
<td>Increased levels</td>
<td>Proforms &lt;br&gt;α-tryptase &lt;br&gt;β-tryptase</td>
<td>Systemic mastocytosis &lt;br&gt;Haematological neoplasms</td>
</tr>
<tr>
<td>Activated</td>
<td>Mature β-tryptase &lt;br&gt;Proforms α-tryptase &lt;br&gt;β-tryptase</td>
<td>Anaphylactic reaction &lt;br&gt;Severe or fatal systemic reactions</td>
</tr>
</tbody>
</table>

*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.*

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**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.*

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

**Risk patients with severe reactions to insect venoms and drugs**
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 anaesthetics 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.
**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.

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


**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**

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### Table: Tryptase profile over time

<table>
<thead>
<tr>
<th>Time</th>
<th>Tryptase profile µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.17 h</td>
<td>57</td>
</tr>
<tr>
<td>3 h</td>
<td>38</td>
</tr>
<tr>
<td>6 h</td>
<td>18</td>
</tr>
<tr>
<td>24 h</td>
<td>5</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Time</th>
<th>Tryptase profile µg/l</th>
</tr>
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<tbody>
<tr>
<td>0.5 h</td>
<td>10</td>
</tr>
<tr>
<td>1 h</td>
<td>9.5</td>
</tr>
<tr>
<td>3 h</td>
<td>10.9</td>
</tr>
<tr>
<td>6 h</td>
<td>10</td>
</tr>
<tr>
<td>24 h</td>
<td>2.1</td>
</tr>
</tbody>
</table>

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<table>
<thead>
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<th>Time</th>
<th>Tryptase profile µg/l</th>
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</thead>
<tbody>
<tr>
<td>30 min</td>
<td>180</td>
</tr>
<tr>
<td>90 min</td>
<td>69</td>
</tr>
<tr>
<td>“next few hours”</td>
<td>“decreased further”</td>
</tr>
<tr>
<td>1 month</td>
<td>17.3</td>
</tr>
<tr>
<td>1 – 18 months</td>
<td>12.8 – 17.3</td>
</tr>
</tbody>
</table>

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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.
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 µg/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 µg/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 µg/l and in the more aggressive forms >>200 µg/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.
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)
✦ Myelodysplastische 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


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