LETTER TO THE EDITOR
EDRA
Eur Ann Allergy Clin Immunol Vol 58, N.6, 286-288, 2026

Danilo Villalta[1], Mariaelisabetta Conte[1], Daniela Visentini[2], Matteo Furlani[3], Francesco Ellero[3], Roberta Metani[3], Fiorenza Pesente[2], Paolo Metus[4]

Redefining reference intervals for baseline serum tryptase: found the solution?

[1]Immunology and Allergy, S. Maria degli Angeli Hospital, ASFO, Azienda Sanitaria Friuli Occidentale (ASFO), Pordenone, Italy
[2]Institute of Clinical Pathology, Department of Laboratory Medicine, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine, Italy
[3]Department of Medicine (DMED), University of Udine, Udine, Italy
[4]Clinical Pathology, S. Maria degli Angeli Hospital, Azienda Sanitaria Friuli Occidentale (ASFO), Pordenone, Italy

Key words

Baseline serum tryptase; reference interval; upper reference limit; clinical decision limits.

Corresponding author
Danilo Villalta
S. Maria degli Angeli Hospital
Azienda Sanitaria Friuli Occidentale
via Montereale 24
33170 Pordenone, Italy
ORCID: 0000-0002-8865-4150
E-mail: villaltadanilo@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.398

To the Editor,

tryptase is a serine protease enzyme primarily expressed by mast cells (MCs) and, to a lesser extent, by basophils (1). The constitutive release of tryptase from MCs results in a baseline serum tryptase (BST) level, which remains relatively stable in individuals throughout life. Since BST levels correlate with mast cell load, persistently elevated levels are an important screening tool for the diagnosis of systemic mastocytosis. In contrast, a transient increase in serum tryptase occurs immediately after MC activation, for example, due to an allergic reaction.

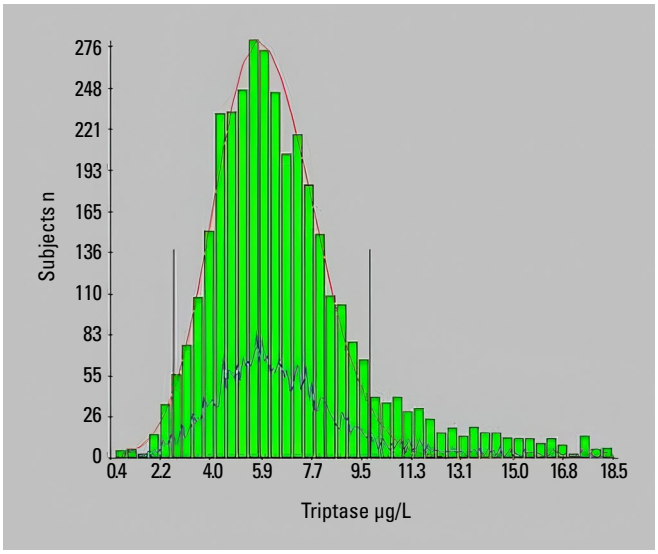
However, severe kidney damage, myeloid neoplasms, certain chronic inflammatory diseases, some chronic infections, and obesity can also cause elevated BST levels (2). Additionally, hereditary alpha tryptasemia (HαT), an autosomal dominant genetic trait characterized by an increased number of *TPSABI* gene copies encoding alpha tryptase, has recently been identified as the most prevalent cause of slightly to moderately elevated BST levels, affecting approximately 4% to 7.5% of the population (3).

Although serum tryptase measurement is performed worldwide using a single fluorescent enzyme immunoassay, there remains ongoing debate regarding the most appropriate reference interval (RI). The generally accepted RI suggested by the manufacturer, based on measuring tryptase levels in 126 'healthy' donors and determining the 95th percentile, is 1.0 (lower reference limit - LRL) to 11.4 µg/L (upper reference limit - URL). While the LRL is not clinically relevant, the URL is highly important for clinical decisions. However, it has been demonstrated that some individuals with systemic mastocytosis may have tryptase levels below 11.4 µg/L (4), indicating that the correct URL is lower than the 11.4 µg/L value proposed by the manufacturer, as the population used to determine the RI undoubtedly included individuals with HαT.

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved

Recently, experts from the European Competence Network on Mastocytosis (ECNM) and the American Initiative in Mast Cell Disease concluded that the "normal serum tryptase range" should be defined in asymptomatic controls, inclusive of individuals with HαT, since HαT *per se* should not be considered a disease-defining feature or pathology. Based on data from the literature, they concluded that the lower limit of BST, below which only a few HαT-positive cases are detected, is 8 µg/L. However, in daily practice, to avoid unnecessary referrals, investigations, and undue concerns in patients with HαT, the "normal range" of basal tryptase is up to 15 µg/L (2). Unfortunately, none of the previous studies in the literature established RIs following the gold standard direct method EP28-A3c (5), suggested by the CLSI (Clinical & Laboratory Standards Institute) and approved by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and by the World Health Organization (WHO). The main limitations of this standard method are that it is expensive, difficult, and time-consuming. An accepted alternative to the direct method is the indirect approach using big data previously generated in the laboratory and stored in the laboratory information system (LIS), referred to as data mining (6). Indirect methods are characterized by the fact that the reference subjects are not selected individually, and the RIs are statistically derived from the total distribution of unselected values. The aim of our study was to obtain the RI of BST using Kairisto's indirect approach, which is based on the assumption that most results produced by the laboratory derive from 'healthy' subjects and hypothesizes that the mode of the frequency distribution is the same for the whole dataset as it is for a 'healthy' reference population. We adopted Kairisto's procedure to correct and standardize the 10.332 tryptase determinations stored in two LIS (Pordenone and Udine, Italy). Only a single value per patient was used, usually the first one. Data were anonymized and elaborated using the GraphROC for Windows, version 2.0 statistical program (7), and mean, mode, LRL (2.5 percentile) and URL (97.5 percentile), were calculated, after the exclusion of outliers, with the use of the iterative ± 4SD method. Mean, mode, LRL and URL values of the whole studied population and divided by age and gender are shown in table 1 and the distribution of tryptase values in whole population in **figure 1**.

Figure 1 *- Distribution of tryptase values in the whole population of the study: 10,332 subjects.*



LRL and URL resulted 2,43 µg/L and 8,96 µg/L respectively, without significant difference between males (2,56 µg/L and 8,64 µg/L) and females (2,8 µg/L and 8,75 µg/L). On the contrary, there was a slight increase with age (**table I**).

The URL of BST obtained with Kairisto's indirect approach is very similar to the value previously proposed by Slot *et al.* (8), who used Bhattacharya's indirect approach on 5,248 measurements, and by the ECNM and the American Initiative in Mast Cell Disease as the "normal range where HαT is rather unlikely" (2). However, in clinical chemistry, the term "normal" range has not been used since Gräsbeck introduced the concept of RI, also known as the reference range (9). RI is a range of values, usually derived from "healthy" or disease-free individuals, to which results can be compared to aid interpretation. It should allow

Table I *- Mean, mode, lower reference limit (LRL) and upper reference limit (URL) obtained with the indirect Kairisto's approach evaluating 10,332 tryptase determinations from two different laboratories.*

	n	Outliers (n)	Mean (SD) µg/L	Mode µg/L	LRL µg/L	URL µg/L
All subjects	10,332	419	5.82 (2.42)	4.75	2.43	8.96
Male	3,699	161	6.04 (2.70)	4.92	2.56	8.64
Female	6,623	268	5.63 (2.26)	3.97	2.80	8.75
≤ 30 Years	2,143	100	4.64 (1.65)	3.67	2.44	7.10
31-60 Years	4,641	209	5.48 (2.04)	5.96	1.74	7.84
>60 years	3,495	163	6.82 (2.88)	5.73	2.70	9.80

the transformation of a number derived from an analytical measurement into clinically meaningful information. On the other hand, clinical decision limits (CDLs) may be an alternative to RIs. CDLs are cut-off values determined by clinical studies and associated with a specific clinical risk or outcome (6). The choice of a CDL is determined by the sensitivity and specificity of a test at that level. While RIs allow for comparison to a reference population, CDLs are linked to clinical outcomes. Regarding BST levels, the RI should be understood as the value obtained using a validated direct or indirect approach, whereas the limits proposed by the ECNM and the American Initiative in Mast Cell Disease should be considered CDLs. For BST, CDLs may be more appropriate than RIs in clinical practice, based on the expert panel's considerations.

In conclusion, the URL of BST obtained with an indirect approach using big data was 8.96 µg/L (ranging from 7.1 µg/L of the subjects < 30 years to 9.8 µg/L of the subject > 60 years). Future prospective studies will be necessary to define the sensitivity, specificity, and positive and negative likelihood ratios for the diagnosis of mastocytosis of the URL we proposed, as well as of the various CDLs suggested by the expert panel. Pending the results of such studies, in clinical practice it is advisable to follow the recommendations provided by the panel of experts.

Fundings

None.

Contributions

DanVill, MC, PM, DanVis: conceptualization, writing – original draft. FP, FE, MF, RM: data collection, formal analysis. All authors: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Vitte J. Human mast cell tryptase in biology and medicine. Mol Immunol. 2015;63(1):18-24. doi: 10.1016/j.molimm.2014.04.001.
2. Valent P, Hoermann G, Bonadonna P, Hartmann K, Sperr WR, Broesby-Olsen S, et al. The normal range of baseline tryptase should be 1 to 15 ng/mL and covers healthy individuals with HαT. J Allergy Clin Immunol Pract. 2023;11(10):3010-3020. doi: 10.1016/j.jaip.2023.08.008.
3. Lyons JJ, Sun G, Stone KD, Nelson C, Wisch L, O'Brien M, et al. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. J Allergy Clin Immunol. 2014 May;133(5):1471-4. doi: 10.1016/j.jaci.2013.11.039.
4. Borer-Reinhold M, Haeberli G, Bitzenhofer M, Jandus P, Hausmann O, Fricker M, et al. An increase in serum tryptase even below 11.4 ng/mL may indicate a mast cell-mediated hypersensitivity reaction: a prospective study in Hymenoptera venom allergic patients. Clin Exp Allergy. 2011;41(12):1777-83. doi: 10.1111/j.1365-2222.2011.03848.x.
5. Boyd JC. CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory: approved guidelines-Third Edition. CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standard institute; 2008.
6. Timbrell NE. The Role and Limitations of the Reference Interval Within Clinical Chemistry and Its Reliability for Disease Detection. Br J Biomed Sci. 2024;81:12339. doi: 10.3389/bjbs.2024.12339.
7. Kairisto V, Poola A. Software for illustrative presentation of basic clinical characteristics of laboratory tests--GraphROC for Windows. Scand J Clin Lab Invest Suppl. 1995;222:43-60. doi: 10.3109/00365519509088450.
8. Slot MC, Claessen LHJ, Bons JAP, Menheere PPCA, Nieuwhof CMG, de Boer D. Tryptase reference ranges are age-dependent in a large population-based cohort. Allergy. 2022;77(9):2833-2834. doi: 10.1111/all.15369.
9. Gräsbeck R. The evolution of the reference value concept. Clin Chem Lab Med. 2004;42(7):692-7. doi: 10.1515/CCLM.2004.118.