LETTER TO THE EDITOR

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

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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 *TPSAB1* 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.

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.

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 1).

The URL of BST obtained with Kairisto's indirect approach is very similar to the value previously proposed by Slot MC. et al (8), who used Bhattacharya's indirect approach on 5248 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 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.

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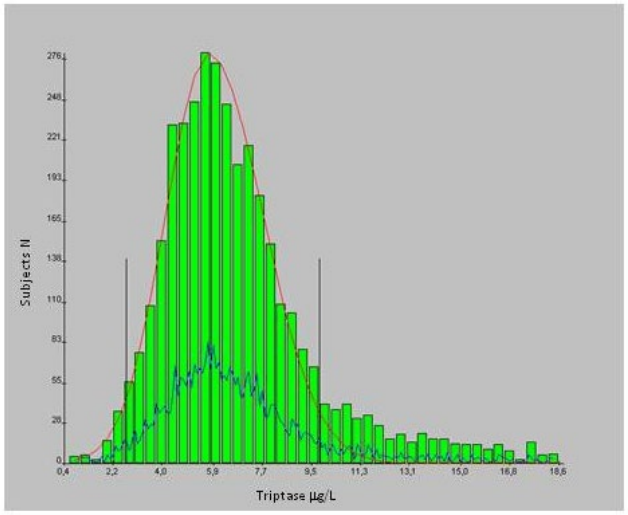
References

1. Vitte J. Human mast cell tryptase in biology and medicine. Mol Immunol 2015; 63: 18-24.
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 2022; 11:3010-20.

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 abnormatilies. J Allergy Clin Immunol 2014;133:1471-4.
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; 41:1777-83.
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; Feb 28:81:12339.
7. Kairisto V, Poola A. Sofware for illustrative presentation of basic clinical characteristics of laboratory tests: GraphROC for window. Scand J Clin Lab Invest 1995; 55(suppl 222):43-60.
8. Slot MC, Claessen LHJ, Bons JAP, Menheere PPCA, Nieuwhof CMC, de Boer D. Tryptase reference ranges are age-dependent in a large population-based cohort. Allergy 2022; 77:2833-4.
9. Gräsbeck R. The evolution of the reference value concept. Clin Chem Lab Med 2004;42. 692-7.

Figure 1. Distribution of tryptase values in the whole population of the study: 10.332 subjects



	N	Ouliers (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

Table 1: 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.