Caveat Emptor – hidden pitfalls in defining the 99th percentile of cardiac troponin assays.

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Defining the criteria for determining the 99th percentile for a cardiac troponin (cTn) assay remains challenging, as the value of the 99th percentile will have a profound influence on whether an assay will achieve the definition of high sensitivity (hs) (1;2).

A previous study demonstrated the effect of population selection on the 99th percentile value (3) . Starting with a random sample of ambulant patients from primary care, selection was based on a health questionnaire (demographics, past medical history especially previous cardiovascular disease, current medication) then surrogate biomarkers of underlying disease (HbA1c, glucose, creatinine and B type natriuretic peptide) physical examination and cardiac imaging (electrocardiogram, echocardiography) (3). Progressive selection (no previous medical history, cardiac medication, then normal physical examination, surrogate biomarkers, physical examination, imaging) produced a shift of the 99th percentile to a lower value high sensitivity assays, the Roche Diagnostics high sensitivity cardiac troponin T assay (hs-cTnT) and a prototype Beckman Coulter hs-cardiac troponin I (hs-cTnI) assay. These findings have been confirmed by other workers (4). Sex specific upper reference limits (URLs) are now recommended (5). A recent publication has caused us to examine patient selection on the hs cTnT assay according to sex (6).

Patient selection reduced total numbers from 545 (99th percentile 30 ng/L) to 340 (questionnaire selection, 99th percentile 20 ng/L) to 200 (imaging plus biomarkers, 99th percentile 14 ng/L). In the baseline cohort 53.5% of females and 62.5% of males had a hs-cTnT > 3 ng/L (respectively 33.6% and 45.6% > 5 ng/L). Following questionnaire selection this reduced to 42.1% of females and 56.1% of males with cTnT > 3 ng/L (respectively 21.3% and 36.9% > 5 ng/L). Selection based on imaging and biomarkers reduced this further to 40.5% in females and 52.4% in males (respectively 19.8% and 35.7% > 5 ng/L). Although there was a reduction due to exclusion of values in the higher end of the tail of the distribution (> 14 ng/L, 4.5%), the majority of the exclusions occurred in the range 3-14 ng/L with an initial 22% of exclusions in females rising to 32.5% with combination of imaging plus biomarkers. This was accompanied by a shift in the distribution curve for the upper half of the data towards a more normal configuration. The results in males are similar to the recently published study (6), although the detection rate is higher in females. Patient selection therefore has a much more marked effect on the percentage detection rate for the Roche hs-cTnT method in females as the majority of the hs-cTnT values in females are lower. This was not seen for the hs-cTnI method. The detection rate was 98.3% in the unselected cases falling to 96.6% based on imaging and biomarker exclusions. A similar performance was seen for the ET Healthcare Pylon hs-cTnT assay used as part of the recent universal sample bank study (6). In females a low detection limit is desirable to achieve high sensitivity performance. Whether or not an assay will detect >50% of the population in question can also be determined by comparing the median value for cTn obtained from the population sample with the limit of detection of the assay. For the data in our study for hs-cTnT, the medians in the unselected population were: 4.7 ng/L in males, 3.3 ng/L in females; 3.6 ng/L in males and <3 ng/L in females in the questionnaire selected group and 3.1 ng/L in males and <3 ng/L in females in the imaging and biomarker selected group,.

In addition to the effect of patient selection on percentage detection and the value of the 99th percentile, shifts in the 99th percentile may have another effect depending on the imprecision profile of the assay. This is illustrated in Figure 1. If the imprecision profile of the assay changes significantly at values close to the 99th percentile, more rigorous patient selection may move the 99th percentile so that it is less than the 10% CV.

Finally, there are pitfalls with the statistical methods used. First, the statistical technique used will affect the value obtained for the 99th percentile (7) as well as the methods used for outlier exclusion (5;8). Second, the recommendation for statistical method is that the 99th percentile be derived from the upper tail of the data distribution, using a nonparametric or robust statistical method (2). When using a nonparametric statistical test from a statistics package, it is important to select the appropriate parameters. When the options of a one-tailed or two-tailed tests are offered then a one-tailed test should be selected, and this should be the upper reference limit only. The percentage selected should be 99%. If both upper and lower reference limits are selected together with a 99th percentile reference limit, the package will calculate the 0.5% and 99.5% reference limits. This is because it will calculate the limits based on 99% of the values and only those exceeding the 99% (the lower 0.5% and the other 99.5%) excluded. When calculating using an upper and lower reference limit, 98% reference limits must be specified to provide 1% below and 1% above the percentiles required, the 1% and 99% values. This is exactly analogous to what is seen with a 95% reference interval where 2.5% are below and 2.5% above the reference limits of the population. Using data taken from our study for the hs-cTnI assay, the 99 percentile upper reference limit when upper and lower values are used for was 47 ng/L. However, specifying a single upper reference for the 99the percentile upper reference limit, the value is 39 ng/L. Similarly, specifying both upper and lower limits calculated for a 98% reference limit, the upper reference limit is similarly 39 ng/L. We conclude, statistician beware.

Criteria for patient selection based on a health questionnaire and markers of occult disease and ventricular function are proposed by the International Federation of Clinical Chemistry committee on Clinical Applications of Cardiac Biomarkers together with the Academy of the American Association of Clinical Chemists (2;5). Wider debate and discussion would be welcomed.

References

1. Sandoval Y, Apple FS, Saenger AK, Collinson PO, Wu AHB, Jaffe AS. 99th Percentile Upper-Reference Limit of Cardiac Troponin and the Diagnosis of Acute Myocardial Infarction. Clin Chem 2020;66:1167-80.

2. Apple FS, Jaffe AS, Collinson P, Mockel M, Ordonez-Llanos J, Lindahl B et al. IFCC educational materials on selected analytical and clinical applications of high sensitivity cardiac troponin assays. Clin Biochem 2015;48:201-3.

3. Collinson PO, Heung YM, Gaze D, Boa F, Senior R, Christenson R, Apple FS. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. Clin Chem 2012;58:219-25.

4. Koerbin G, Abhayaratna WP, Potter JM, Apple FS, Jaffe AS, Ravalico TH, Hickman PE. Effect of population selection on 99th percentile values for a high sensitivity cardiac troponin I and T assays. Clin Biochem 2013;46:1636-43.

5. Wu AHB, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Llanos J, Apple FS. Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem 2018;64:645-55.

6. Apple FS, Wu AHB, Sandoval Y, Sexter A, Love SA, Myers G et al. Sex-Specific 99th Percentile Upper Reference Limits for High Sensitivity Cardiac Troponin Assays Derived Using a Universal Sample Bank. Clin Chem 2020;66:434-44.

7. Eggers KM, Apple FS, Lind L, Lindahl B. The applied statistical approach highly influences the 99th percentile of cardiac troponin I. Clin Biochem 2016;49:1109-12.

8. Hickman P.E., Koerbin G., Potter J.M., Glasgow N., Cavanaugh J.A, Abhayaratna W.P et al. Choice of Statistical Tools for Outlier Removal Causes Substantial Changes in Analyte Reference Intervals in Healthy Populations. Clin Chem 2020;66:1558-61.

Figure 1. Effect of population selection on assay designation according to assay imprecision profile for two hypothetical cardiac troponin assays. Progressive reduction of the 99th percentile from 30 ng/L to 10 ng/L has little effect on assay 1 which has a 10% imprecision below 5 ng/L. Assay 2 becomes borderline at 20 ng/L but fails to reach the target of <10% imprecision for a 99th percentile of 10 ng/L so would switch designation from high sensitivity to contemporary sensitive.