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Analysis of patient results distributions to reevaluate a reference range change for calcium, after a change in assay reagents on the Roche Cobas c500 analyzer

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INTRODUCTION

A change in reagents for calcium (Ca) on the Roche Cobas c500 used in our laboratory analyzer took place in 2013. The previous reference range (8.5-10.5 mg/dL) was replaced with that from the manufacturer's study (8.6-10.0 mg/dL), based on correlation of results between the new and old assays. As a matter of quality assurance, we undertook a post-assay-change reevaluation of the reference range change, using a method based on that of Bhattacharya [1]. In short, the method relies on the assumption that the reference range is a normal distribution, which assumption enables this distribution to be isolated mathematically from within all-comers patient distribution data that are not normally distributed.

METHODS AND RESULTS

Primary data were all patient Ca results retrieved for a one-month interval (**Figure 1)**.

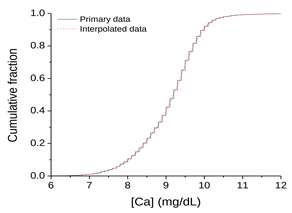

Figure 1. Primary data: cumulative results distribution for all patient Ca results retrieved for a one-month interval (January, 2014; n = 11,684). Solid line: distribution according to 0.1 mg/dL increments of reporting. Dashed line: continuous data distribution interpolated from original data. Vertical dashed lines: boundaries of reference range (8.5-10.0 mg/dL). For this distribution, low Ca = 24.9%, high Ca = 9.2%.

Isolation of the data subset compatible with a normal distribution was a two-stage process: The point of maximum slope of the cumulative patient results distribution was determined to define the mean/median of the embedded normal distribution (9.4 mg/dL; **Figure 2**).

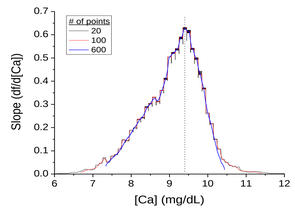

Figure 2. Slope of the continuous cumulative patient results distribution vs. Ca. Maximum slope was centered at Ca = 9.4 mg/dL, which was then defined as the normal distribution midpoint/median.

For each of these intervals, an iterative search was made to determine the central fraction of a normal distribution encompassed by each interval, as evidenced by the linearity of a normality plot when the correct fraction was specified (**Figure 4**).

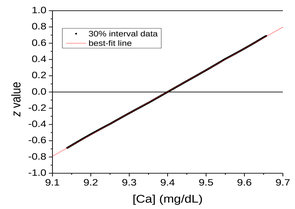

Figure 4. Example of normality plot. For interval = 30% of results centered on the midpoint (i.e., midpoint ± 15% of results), the maximum linearity of the normality plot (r^2 >0.999) occurred when assuming that this interval was inclusive of 51% of a normal distribution. Correspondingly, the associated reference range was 8.65-10.14 mg/dL. Normality plot: x-axis = Ca result (mg/dL); y-axis: *z* value (-∞ to +∞) based on assumed percentile of results within the normal distribution.

Varying widths of intervals of results having symmetry around this midpoint (a necessary condition for a normal distribution) were assigned for analysis (**Figure 3**).

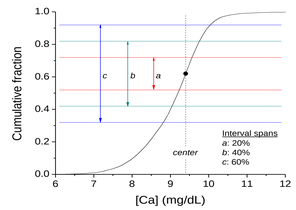

Figure 3. Examples of intervals of results having symmetric percentages of results on either side of the defined midpoint.

Results of these procedures converged on a reference range for Ca of 8.6-10.2 mg/dL (**Figure 5**)..

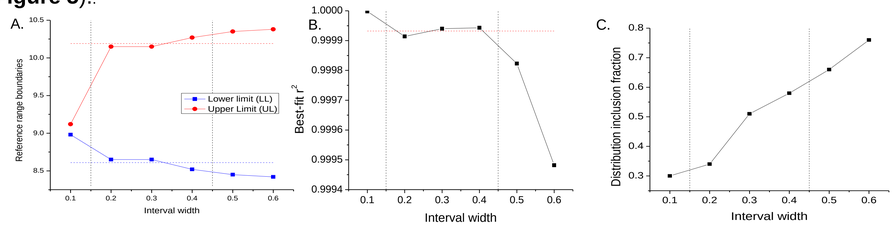

Figure 5. Results of best-fit normality plots as a function of assumed interval widths. A. Upper limit (UL) and lower limit (LL) of reference ranges. B. Linear correlation coefficient (r^2) of best-fit. C. Percent inclusion of normal distribution within interval for best-fit. Vertical dashed lines: boundaries of region of converging analyses (based on r^2). Horizontal dashed lines (A): average UL and LL based on converging analyses.

The reference range from Figure 5 data was essentially identical (±0.1 mg/dL) to "textbook" reference ranges (e.g., [2]). A comparison of the patient results distribution to the reference range distribution is shown in **Figure 6**.

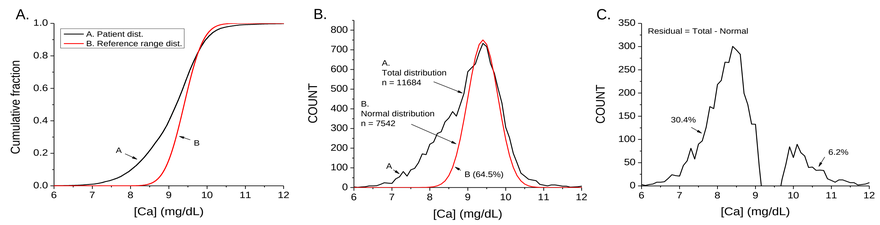

Figure 6. A. Comparison of patient results cumulative distribution to reference range cumulative distribution. B. Comparison of patient results distribution to reference range distribution. C. Residuals between patient results distribution and normal patient distribution (residual = patient distribution - reference range distribution).

CONCLUSIONS

The results were used to update our Ca reference range. Normal distribution analysis of patient data subsets by this method can be a powerful tool to evaluate reference ranges, simply because it can include a large number of patients using retrospective data. In comparison, identification and testing of "normal" patients in similar numbers would be difficult or impractical. In particular, clinical verification of a normal population for Ca would be expensive for any large number of patients, involving combined evaluation of Ca, renal function, vitamin D status, and PTH. These results demonstrate that one can have reasonable confidence in an esoteric method for extraction of a reference range from an all-comers patient results distribution. The related method of Hoffman [3] is more well-known but less stringent, being applied with varying degrees of success in recent literature [4-7].

REFERENCES

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