An Approach for the Development of Reference Intervals for Platelet Aggregation Testing.

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Platelet aggregation testing is important to evaluate platelet function disorders. Historically, reference intervals (RI) for percentage aggregation responses are derived using the mean ± 2 SD (MSD) for healthy volunteer samples. However, a nonparametric approach (NP) is acceptable for RI determination, especially if there is more than one observation per individual or non-normal distribution of data. We compared MSD and NP approaches to determine platelet aggregation RI.

Methods: Data on % aggregation by light transmission was prospectively collected for platelet rich plasma (250 x 10^9 platelets/L) from healthy controls (some tested multiple times), stimulated with (final concentrations): 2 and 4 μM ADP, 1.25 and 5 μg/mL Horm collagen, 1.6 mM arachidonic acid, 1 μM thromboxane analogue, and 0.5 and 1.25 mg/mL ristocetin. RI were estimated by MSD and NP using "all" data and "first" measurements, with 2.5% of data outside lower or upper RI considered acceptable.

Results: As the % aggregation responses to most agonists had non-normal distributions, RI by MSD was not appropriate particularly for ADP and low dose ristocetin which showed dramatic deviations from a normal distribution. The use of all or first measurements generated similar RI by NP but not by MSD. In addition, NP RI were not dependent on data distribution, resulting in more acceptable proportions of values above or below RI than with MSD (% above or below RI using "all" ; "first" measurements: NP 0.94-2.21%; 1.33-2.13%, MSD 0.88-6.36%; 0.00-5.52%).

Conclusion: RI determination by a nonparametric approach generated the most representative RI for % aggregation and it had the advantage of using all available repeated measurements, without dependency on data distribution. Because the nonparametric approach is also recommended when only a limited numbers of controls are tested, we suggest that it be the preferred approach for establishing platelet aggregation RI.