Original Article

Determination of reference intervals from a laboratory database of an academic clinical research unit in a tertiary care teaching hospital and an audit of out of range values

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Abstract

Background: Abnormal laboratory values are a common reason for the exclusion of participants in clinical studies, increasing the recruitment time and cost during conduct. The use of sample-specific reference intervals (RIs) may help to address this issue. Hence, the present study derived site-specific RIs using the department laboratory database and compare the proportion of "out of range" (OOR) values between the new and the old RIs used by the trial site.

Methods: Institutional ethics committee approval was obtained. Data for hematology and biochemistry parameters were analyzed. Normality was assessed and RIs computed using nonparametric method. Data were partitioned for gender and descriptive statistics applied for demographics. The OOR values based on new RIs were compared with old RIs using Chi-squared tests. Between gender OOR proportions compared using Chi-squared test (significance at $P < 0.05$). *Post hoc* analysis was performed with Beasley's technique.

Results: Data of 601 participants were analyzed. The median (Inter Quartile Range) age was 22 (47) years and 64.72% were male. New RIs for key parameters were: Haemoglobin (9.3–16.5 g/dl), alanine aminotransferase (11.4–47.74 U/l), aspartate aminotransferase (8.8–58 U/l), total bilirubin (0.27–1.4 mg/dl), and creatinine (0.59–1.36 mg/dl). Post partitioning, the RI for hemoglobin (g/dl) was lower (8.72–15.72) in females. The proportion of OOR values were lower with new RIs relative to old laboratory RIs ($P < 0.0001$).

Conclusion: A reduction in the proportion of OORs and a change in the upper and lower bound laboratory intervals with new RIs emphasize the need for sample-specific ranges to prevent unnecessary exclusions of volunteers from trials.

Keywords: Clinical trials, exclusions, out of range values, reference intervals

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INTRODUCTION

Recruiting normal healthy participants for research studies is both challenging and arduous.[1] Participants are considered "normal" if their laboratory values fall within preassigned reference intervals (RIs).[2] RIs are used to exclude participants with subclinical illnesses who are at high risk for adverse events (AEs) or whose participation could confound the interpretation of study results. On the other end, differentiating these AEs from the expected physiological/diurnal variations affecting the parameters is equally important.[3,4]

RIs are defined by testing a representative sample from the population under study and calculating the 95% confidence limits specific to that population.[5] Determination of RIs is a difficult, time-consuming, and expensive process and many laboratories adopt and use RIs from literature. This tends to overlook populations studied, analytical methods and instruments used for deriving them.[5] Abnormal laboratory values are a common reason for screen failures in studies[6,7] and could be the result of the use of RIs that are not representative of the population screened for the trial.

Hence, the objective of the present study was to derive RIs specific to our site using an existing laboratory database of values. The secondary objective was to assess the proportion of "out of range" (OOR) values based on the newly derived RIs. Further, we proposed to compare these OOR values with OOSs based on RIs of two national accreditation board for laboratories (NABL) accredited laboratories and the old RIs of our department laboratory.

METHODS

Ethics

Institutional Ethics Committee approval was taken (EC/0A-10/2015), who granted a consent waiver.

Study site

The research department in a tertiary care teaching hospital.

Steps involved in the calculation of reference intervals

The Clinical and Laboratory Standards Institute (CLSI) Guidelines[5] were used for calculation of RIs.

Study sample

Anonymized and coded data for hematology and biochemistry parameters for $n = 603$ participants were assessed.

Eligibility criteria

Healthy adults between the age group of 18–65 years and screened between the years 2010 and 2016 for clinical trials were included in the study.

Computation of reference intervals

A nonparametric approach based on computing the upper and lower bound values of the RIs as 97.5 percentile and 2.5 percentile was used[5] in view of nonnormally distributed data.

Partitioning criteria

We used gender as the partitioning criterion and stratified the RIs separately for males and females.

Out of range values

These were analyzed based on New RIs, RIs of two NABL accredited laboratories (Lab 1 and 2) and the old RIs of our department (Clinical Pharmacology) laboratory.

Data management

All data were entered into Microsoft Excel (Publisher: Microsoft Corporation, Redmond, Washington, USA, 2016) and coded.

Statistical analysis plan

Demographic data were summarized using median and interquartile range (IQR). The proportion of OOR values based on the new RIs were compared with those of comparator laboratories. *Post hoc* analysis was done as per Beasley's technique, which is a *post hoc* method that uses multiple regression to interpret Chi-square contingency tables.[8] Multiple pairwise comparison between the groups, an alpha correction (Bonferroni's correction)[9] using the formula $1 - ([1 - \text{alpha}]^{1/t})$ was applied to determine significance, where "t" denotes the number of groups to be compared. Substituting $t = 3$ and alpha = 0.05, the new level of significance is $P = 0.017$. The number of OOR values between males and females was compared using Chi-squared test. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using MedCalc version 17.2 (Publisher: Medcalc Software, Ostand, Belgium, 2017), Graphpad Prism Instat version 5 (Publisher: Graphpad Software, La Jolla, California, USA, 2007) and Statistical Package for Social Sciences (SPSS) for Windows, version 20.0 (Publisher: IBM, Armonk, New York, USA, 2011).

RESULTS

Demographics

Of the $n = 603$ participants, gender details were unavailable for two participants and $n = 601$ formed the final sample. The median (IQR) age was 22 (47) years. A total of 153/601 (25.37%) were screened for interventional studies, while 448/601 (74.54%) were screened for observational studies.

The newly formulated reference intervals

The newly derived RIs differed from the reference laboratories with respect to both upper and lower bound values. We observed that for hemoglobin the lower bound value (in g/dl) was below ten (9.3) as per new ranges; in contrast, it was 13.5 for NABL accredited labs and 11 as per the Department of Clinical Pharmacology (DCP) old ranges. The upper bound values for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in U/I as per new ranges were 48.90 and 61.60 respectively which were higher relative to the DCP and the NABL accredited laboratories that had values lower than 42U/I. Similarly, for total bilirubin, the upper bound value (in mg/dl) as per the new range was 1.4 relative to 1.2 seen for the DCP laboratory and 1.0 as seen for the NABL accredited laboratories. The details of the RIs for all parameters are summarized in Table 1.

Reference intervals post partitioning based on gender

The lower bound value for hemoglobin in females (8.72–15.72 g/dl) was much lower than those seen in males (10.37–16.50 g/dl). While for liver function tests, the upper bound values for both AST (39.72 U/I) and ALT 40.96 U/I) were slightly higher in males relative to females. Details are summarized in Table 2.

Out of range values after derivation of new reference intervals

The total proportion of OOR values were between 0.16%–4.53%. A significant reduction in OORs was seen for hemoglobin for new intervals (4.18%) relative to that seen for NABL laboratories (54%) and old department RIs (47%). In biochemistry parameters a lower proportion of OOR (2.81%) was seen for creatinine with new intervals relative to that seen for NABL accredited laboratory 1 (80%). Details are given in Table 3.

The proportion of OOR values were significant between the laboratory RIs for all parameters except platelet count ($P = 0.04$) and neutrophils ($P = 0.40$). A *Post hoc* analysis showed that the proportion of OORs

Table 1: Reference intervals computed as per nonparametric method from the database

Parameter	Number of participants in the database	New RI	Old RI of department lab	NABL accredited Lab-1 RI	NABL accredited Lab-2 RI
Haemoglobin (g/dl)	597	9.3-16.5	11-18	13.5-18	13.5-18
Platelet count (per µl)	396	165-421	150-400	150-400	150-450
WBC (per µl)	395	4.1-10.7	4.5-10.5	4.5-11	4-10.5
RBC (per µl)	321	4-6	4-6	4.6-6.2	4.7-6
Lymphocytes (%)	596	18-47	20-40	20-45	20-40
Eosinophil (%)	544	0-7	2-10	2-10	2-10
Neutrophil (%)	598	46-78	40-80	40-75	40-80
Monocytes (%)	598	1-10	2-10	2-10	2-10
Basophils (%)	596	0-1	0-2	0-2	0-2
BUN (mg/dl)	581	5.7-18.2	6-21	5-18	8.9-20.6
Creatinine (mg/dl)	593	0.6-1.4	0.7-1.4	0.4-0.7	0.7-1.3
Total bilirubin (mg/dl)	554	0.3-1.4	0.1-1.2	0-1.0	0.2-1.2
AST (U/I)	579	8.8-58	Up to 37	Up to 40	Up to 45
ALT (U/I)	575	11.4-47.74	Up to 42	Up to 41	Up to 35
Alkaline phosphatase (U/I)	331	22-133	15-112	0-299	Not available

Lower and upper bound intervals the 2.5 and 97.5 percentile respectively. WBC=White blood corpuscles, RBC=Red blood corpuscles, BUN=Blood urea nitrogen, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, NABL=National Accreditation Board for Laboratories

Table 2: Reference intervals derived for males and females from the database

Parameter	Number of males (*n*)	RIs for males	Number of females (*n*)	RIs for females
Haemoglobin (g/dl)	387	10.4-16.5	210	8.7-15.7
Platelet count (per µl)	272	167-401	124	174.8-398
WBC (per µl)	270	4.1-10	125	4-10.2
Lymphocytes (%)	387	17.9-47	209	19.2-46
Eosinophil (%)	341	0-3	203	0-3
Neutrophils (%)	387	46-78	211	48-–78
Monocytes (%)	387	1-10.4	211	1-7.6
Basophils (%)	386	0-1.4	210	0-0
BUN (mg/dl)	374	6-15.4	207	5.4-15.2
Creatinine (mg/dl)	381	0.64-1.4	212	0.58-1.2
Total bilirubin (mg/dl)	345	0.27-1.4	209	0.32-1.3
AST (U/I)	372	11.4-39.7	207	11.4-37
ALT (U/I)	366	8.8-41	209	7.2-35.7

For RBC and alkaline phosphatase since the per subgroup values were less than 120, RIs were not computed. WBC=White blood corpuscles, RBC=Red blood corpuscles, BUN=Blood urea nitrogen, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, RIs=Reference intervals

Table 3: OOR values compared between reference intervals of the comparator laboratories (DCP, National Accreditation Board for Laboratories accredited Laboratory 1 and 2)

Parameter	*n*	OOR as per new RI, *n* (%)	OOR as per old RI of department, *n* (%)	OOR as per laboratory 1, *n* (%)	OOR as per laboratory 2, *n* (%)	Actual *P*
Haemoglobin*	598	25 (4.18)	78 (13.04)	326 (54.51)	326 (54.51)	<0.00001
Platelet count	401	14 (3.49)	16 (3.90)	16 (3.90)	4 (0.99)	0.04
WBC†	401	13 (3.24)	31 (7.73)	28 (6.98)	12 (2.99)	0.002
RBC*	317	25 (7.88)	25 (7.88)	121 (38.17)	143 (45.11)	0.00001
Lymphocytes*	598	26 (4.34)	104 (17.39)	47 (7.85)	104 (17.39)	0.00001
Eosinophil‡	598	1 (0.16)	33 (5.51)	33 (5.51)	33 (5.51)	0.00001
Neutrophil	598	4 (0.67)	1 (0.16)	3 (0.50)	0 (0.00)	0.4
Monocyte‡	598	11 (1.83)	31 (5.18)	7 (1.17)	0 (0.00)	0.00003
Basophil	596	13 (2.18)	0 (0.00)	26 (4.36)	0 (0.00)	NA
BUN*	601	16 (2.66)	26 (4.32)	5 (0.83)	247 (41.09)	0.00001
Creatinine*	603	17 (2.81)	36 (5.97)	505 (83.74)	54 (8.95)	0.00001
Total bilirubin†	556	7 (1.25)	265 (47.66)	20 (3.59)	25 (4.49)	0.00001
AST$	602	14 (2.32)	26 (4.31)	9 (1.49)	10 (1.66)	0.0053
ALT†	603	17 (2.81)	32 (5.30)	14 (2.32)	44 (7.29)	0.00004
Alkaline phosphatase$	331	15 (4.53)	18 (5.43)	1 (0.30)	NA	0.0005

*Significant for all 3 comparisons ($P<0.0001$), †Not significant for comparison of proportion of OORs based on new RI versus old RI of our lab, but significant for comparison with OOR based on both outside lab RIs, ‡Not significant for comparison of proportion of OORs based on new RI versus old RI of our lab and for new RI versus Lab-1 RI, but significant for comparison of OOR based on new RI versus lab-2 RI, $Significant for comparison of proportion of OORs based on new RI versus old RI of our lab, but not significant for comparison with OOR based on both outside lab RIs. OOR=Out of range, RIs=Reference intervals, WBC=White blood corpuscles, RBC=Red blood corpuscles, BUN=Blood urea nitrogen, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, DCP=Department of Clinical Pharmacology

Table 4: Nominal *P* values (defined as *P* value obtained after the post hoc chi square analysis) comparator laboratories (after applying Bonferroni correction)

Parameter	Nominal *P* values after applying Bonferroni correction		
	OOR based on new RI versus old institute RI	OOR based new RI versus Lab 1 RI	OOR based on new RI versus Lab 2 RI
Haemoglobin (g/dl)	<0.017*	<0.017*	<0.017*
WBC (per µl)	0.3173	<0.017*	<0.017*
RBC (per µl)	<0.017*	<0.017*	<0.017*
Lymphocytes (%)	<0.017*	<0.017*	<0.017*
Eosinophil (%)	0.0357	0.0719	<0.017*
Monocyte (%)	0.3200	0.2700	<0.017*
BUN (mg/dl)	<0.017*	<0.017*	<0.017*
Creatinine (mg/dl)	<0.017*	<0.017*	<0.017*
Total bilirubin (mg/dl)	<0.017*	<0.017*	<0.017*
AST (U/l)	<0.017*	0.0455	<0.017*
ALT (U/l)	0.0214	<0.017*	<0.017*
Alkaline Phosphatase (U/l)	<0.017*	0.0214	0.0214

*$P<0.0062$. OOR=Out of range, RI=Reference interval, WBC=White blood corpuscles, RBC=Red blood corpuscles, BUN=Blood urea nitrogen, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase

based on new RIs for hemoglobin, lymphocytes, red blood cell (RBC), blood urea nitrogen and creatinine were significantly different from those of the three comparator intervals ($P < 0.0001$), the new statistical significance being $P < 0.0062$ after Bonferroni's correction) Table 4.

Out of range values between males and females

The proportion of OOR values were higher in males for total bilirubin (12/386, 3.10% vs. 2/212, 0.94% in females, $P = 0.16$) and neutrophils (17/386, 4.40% vs. 08/204, 3.92% in females, $P = 0.02$) respectively. Among females, higher OOR values were seen for hemoglobin (13/212, 6.13% vs. 11/386, 2.84% in males, $P = 0.08$).

DISCUSSION

In the present study, we derived site-specific RIs for our laboratory using the existing participant database. We found that the RIs were quite different from the comparator laboratories. Females particularly had lower values of hemoglobin relative to the males and there was a reduction in the number of individuals who presented with OOR values based on the new RIs. RIs are an important decision-making tool for screening and enrolment of participants in trials from a representative population.[9] An earlier study from our center showed that a majority of participants (74%) were excluded due to the so-called 'abnormal' laboratory values. Sibille and Vital Durand in their paper on screening of healthy participants emphasized the need to redefine

"laboratory normal ranges as a function of the population being investigated."[4] Defining sample-specific RIs while screening may help sites develop ethical approach and prevent unnecessary exclusions during screening.

The CLSI guideline states that consideration of physiological and preanalytical factors is important when deriving sample-specific reference ranges. The laboratory RIs for hemoglobin, AST and ALT observed by us are different from the RIs of the comparator laboratories while those for white blood cell, RBC, neutrophil, and basophil are overlapping. The RI for hemoglobin is largely driven by the lower values seen in females, who tend to have a lower hemoglobin value than the World Health Organisation (WHO) recommended criteria.[10] Also, most of the participants who present to our hospital come from a lower socioeconomic stratum, and factors like illiteracy and poverty associated with lower-income groups may contribute to anemia or low hemoglobin levels.[11,12]

In the case of the liver function tests, we found that the upper bound values in newly derived RIs were higher relative to the RIs of the comparator laboratories. Our observation is similar to a study by Furruqh *et al*., Yadav *et al*. and Shah *et al*. in India who reported a wide range for liver function tests with a higher upper bound value.[13-15] The upper limit of normal seen with the ALT values could be due to the divergent characteristics of the cohorts that are used by the individual laboratories.[16] When gender-specific RIs were derived, we found that the RI for hemoglobin (mg/dl) in females (8.7–15.7) had a lower bound that was much lower relative to men (10.4–16.5). Our result is consistent with the observation of Ashavaid *et al*. who reported that the lower limit of hemoglobin for nonpregnant adult females in India was 1 g/dl less than the WHO and Institute of Medicine criteria.[10] Another study by Sairam *et al*. also observed a gender-specific variation for hemoglobin with lower levels seen in females.[17]

The proportion of OOR values ranged between 0.16% and 4.56% and were significant for all except platelet count and neutrophil. Physiological and preanalytical factors can impact laboratory values. For example Gender in case of hemoglobin and prolonged fasting, Gilbert's syndrome in case of bilirubin,[18,19] and therefore, must be considered to avoid unnecessary exclusions. Participants without any subclinical illness are defined as "Clinically normal," at the same time, those participants whose laboratory values are outside the 95% confidence limit (as per the guideline) are called as "statistically normal." Population specific RIs in trials could help reduce exclusions of "*Clinically normal*" participant on account of "*Statistically abnormal*" laboratory values.

The present study is limited by being conducted at a single center and has a retrospective design. The RIs merely indicates the central 95% of the sampled population and interval can change over time (drift) as analytical systems evolve and must be revised.[20]

CONCLUSION

A reduction in the proportion of OORs with new RIs and variation in the upper and lower bound laboratory values emphasize the need to have sample-specific intervals to prevent unnecessary exclusions of volunteers from trials.

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Conflicts of interest
There are no conflicts of interest.

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