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Use of Sigma Metrics in the Evaluation of Analytical Performance of Apo lipoproteins A1 and B Analytes

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Abstract: ***Background: Standardization of Cardiovascular risk marker measurements is an essential prerequisite for improving cardiac health. Six Sigma metrics, is used to assess the analytical quality of automated clinical chemistry parameters in clinical laboratory and to explore the importance of the source used for estimation of the allowable total error. Analytical performance and quality specifications were evaluated for the parameters of Apo A and Apo B parameters in cardiovascular risk patients to assess the risk in this study. Method: Coefficient of Variation (CV%) and External Quality Assurance Scheme (EQAS) bias% data for Apolipoprotein A and Apolipoprotein B analytes were collected for the year 2022 for a period of 6 months. TEa calculated for each analyte was calculated based on average CV% and bias%. Total TEa calculated values are compared with optimal, minimal and desirable TEa of each analyte. Six Sigma Score is evaluated for Apo A & Apo B parameters against Minimum, Desirable & Optimum quality specifications. Results: Average CV % is within acceptable limits as per Desirable & Optimum specifications whereas not acceptable as per Minimum specifications. Percentage bias is within acceptable limits as per all 3 specifications. TEa is within acceptable limits as per Desirable & Optimum specifications whereas not acceptable as per Minimum specifications. Six Sigma score was acceptable for Apo A under only Optimum quality specification. For Apo B, Six Sigma score was acceptable under both desirable & optimum quality specifications. Conclusion: Sigma metrics is an excellent quality management tool and quantitatively to evaluate analytical performance. The accurate results generated are useful for clinicians for decision making.***

Keywords: Apo lipoprotein A1 and Apolipiprotein B, Coefficient variation (CV), external quality assurance scheme (EQAS), Total allowable error (TEa).

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide and its prevalence is expected to continue to rise over the next 15 years. The risk of coronary events in patients with or without CAD [1, 2] as well as silent myocardial infarction and silent CAD in high - risk patients with type 2 diabetes [3]is on the rise. Additional findings such as elevation of small dense low - density lipoprotein (sd - LDL) particles, Abnormal results of Apo lipoprotein B (Apo - B), Apo lipoprotein a (apo - A) and detection of large TG rich very low - density lipoproteins and oxidized LDL, as well as decreased number of small HDL particles further contributes to cardio vascular risk [4]. According to the American Heart Association (AHA), one in three people will be affected by some form of CVD during their lifetime [5]. The two most common clinical manifestations of CVD are coronary artery disease (CAD) and ischemic stroke.

While ApoB acts as a major transporter for all atherogenic particles, apoA1 is an anti - atherogenic lipoprotein responsible for transporting cholesterol within HDL - C. Sigma metric is used as a quality management strategy for a laboratory process to improve the quality by addressing the errors after identification

Standardization of ApoA1, apoB measurement improves the quality of testing as studies support the benefits of apoB in cardiovascular health assessment [6, 7].

To evaluate Apo A1 & Apo B performance, the analytical imprecision and bias (obtained from the internal quality control protocol) were compared against the quality specifications (standards) for these two components of analytical error.

The study was done by evaluating the imprecision, bias, Total error & six sigma score calculation of Apo A & Apo B in our laboratory.

2. Materials and Methods

This study was done in our reference laboratory in Bangalore. The analytes involved in this study were Apoliprotein A 1 and Apolipoprotein B. All the analytes were processed in the Roche Cobas 8000 analyzer with its dedicated reagents. Bio - Rad Laboratories (Bio - Rad Inc., California, USA), including the following Immunology two levels: the normal level (level 1, lot no: 68991) and high level (level 2, lot no: 68992) were used as internal quality control (IQC) materials. MHL EQAS was done for the interval of 3 months. The methods for detecting Apolipoprotein A and Apo lipoprotein B is immuniturbidometry.

The mean values and standard deviations were calculated for these two analytes ie Apo A1, Apo B the controls were plotted on the Levey Jennings chart to check acceptability according to Westgard rules. Between day imprecision, bias

and (TE) were determined for each analyte on COBAS analyzers as follows -
CV% = (*SD*/Mean) × 100
CV (%) is the coefficient of variation for measuring between day imprecision, SD is the standard deviation
Bias % = (*Average* absolute deviation from the target value/Target) × 100

Referring to formula: TEa = 2*CV%+Bias %. TEa were calculated for each analytes.

The CV data represent the imprecision of each analyte and were derived from IQC (two levels) analysis from January to June 2022. Two levels IQC was run at the morning. Mean and Standard deviation (SD) were calculated. Monthly CV % was calculated by the formula, Highest CV% out of two levels were selected for each month and finally average CV% was calculated.

Bias represents the trueness of each analyte, and it was determined based on EQA samples of Apo lipoprotein A and Apo lipoprotein b in 2022. EQAS report were used for the average absolute value of the above single percentage difference was defined as the bias of that analyte and used for the calculation of its TEa.

Average Bias % was calculated for each urine biochemical analytes. TEa % calculated for each analytes using the above said formula.

Referring to formula: TEa = 2*CV%+Bias %. TEa were calculated for each analytes.
The sigma metrics (Pr) was calculated by the formula:

$$\sum \sigma = TEa - Bias/CV$$

Where, TEa is the total allowable error of analyte taken from BV guidelines from EFLM database (westgard)

3. Results

Table 1: APO A1 and APO B analytes - CV% and bias % data for the year 2022

s. no	Analyte	JAN 2022	FEB 2022	March 2022	Apr 2022	May 2022	Jun 2022
1	ApoA1						
	% CV	2.8	2.5	2.5	2.2	2.0	2.3
	EQAS bias %	0.27	1.01	1.8	1.1	0.09	2.7
2	ApoB						
	% CV	1.8	2.1	2.8	2.1	2.4	2.1
	Eqas % Bias	2.11	2.12	1.42	1.18	1.76	2.9

Table 2: Total Analytical error calculation

	MINIMUM			DESIRABLE			OPTIMUM			Study Result		
Analyte (Matrix: Serum)	Imprecision (%)	Bias (%)	TEa (%) p<0.05	Imprecision (%)	Bias (%)	TEa (%) p<0.05	Imp (%)	Bias (%)	TEa (%) p<0.05	% CVA	Analytical BIAS%	Total Analytical Error
Apo A1	1.3	1.5	3.7	2.7	3.1	7.5	4.0	4.6	11.2	2.35	1.16	5.86
ApoB	1.8	2.7	5.7	3.7	5.3	11.4	5.5	8.0	17.1	2.11	1.91	6.13

Table 3: Sigma Score

	MINIMUM	DESIRABLE	OPTIMUM
	SIGMA	SIGMA	SIGMA
Apo A1	1.1	2.7	4.3
ApoB	1.8	4.5	7.2

4. Discussion

Apo B measurement is superior to LDLC and non - HDLC measurements and calculations for the assessment of exposure to atherogenic lipoprotein particle numbers in the circulation ApoB is recommended for risk assessment and may be preferred over non - HDLC, if available, in persons with mild - to - moderate hypertriglyceridemia (2–10 mmol/L), diabetes, obesity or metabolic syndrome, or very low LDLC <1.8 m mol/L [8]. Like non - HDLC, apo B can always be measured in the non - fasting state and is not affected by biological TG variability.

Although the traditional lipid profile of TC, TG, HDLC, and LDLC remains essential for dyslipidaemia diagnosis and ASCVD risk categorization, the position of LDLC as treatment target is challenged by the analytical performance of Apo B and Apo A. Apo lipoproteins tests are useful for clinical performance, clinical effectiveness, and cost - effectiveness – beyond analytical performance to become a medically useful test for the assessment of cardio vascular risk patients other than lipid profile.

Apo lipoprotein A - I fractional catabolic rate mostly determines circulating HDL levels. ApoA - I and is the major protein moieties of HDL, and approximately 90% of total apo A - I and apoA - II is found within HDL density range. Apo A - I is synthesized in the liver and small intestine, has a molecular weight of 28.000 kDa, and, in association with lipids, antiparallel dimers of apoA - I form an extended "belt" around the periphery of both spherical lipoproteins and bilayer disc complexes with hydrophobic regions of protein in contact with a lipid surface [9].

Apo lipoprotein A1 (ApoA1) is a main proteinmoiety in high - density lipoprotein (HDL) particles. Generally, ApoA1 and HDL are considered as atheroprotective. In prooxidant and inflammatory microenvironment in the vicinity to the atherosclerotic lesion, ApoA1/HDL are subjected to modification. The chemical modifications such as oxidation, nitration, etc result in altering native architecture of ApoA1 toward dysfunctionality and abnormality. (10)

Apo lipoprotein A1 (apoA1) is a principal protein component of HDL. Plasma HDL cholesterol and ApoA1

levels are associated with lower CVD risk, especially MI risk. (11, 12) However, atherosclerosis - related oxidative stress and inflammation lead to oxidation and other modifications of mature ApoA1, a phenomenon that can switch atheroprotective properties of native apoA1 to proatherogenic properties of modified ApoA1. (13) ApoA1 modification could induce formation of ApoA1 - specific IgG antibodies that exhibit pro - inflammatory properties. In this review, we consider ApoA1 structural and functional properties, and a role of ApoA1 - specific antibodies in atherosclerotic disease.

In patients with a moderate estimated risk score, in particular those with additional metabolic risk factors, apo A1, apo B measurement as a "risk - enhancing factor" could be useful.

Biological variation, the natural fluctuation of body fluid constituents around the homeostatic setting point, has two components: within and between - subject variation, It is clear that clinical laboratory performance should satisfy medical needs (14) which include monitoring, screening, diagnosis and case finding. The analytical quality specifications for imprecision, bias and total error can be used in daily work for two different activities:

The between day imprecision, bias and TE for the two instruments were checked for each analyte to see if they were within the limits of minimum, desirable and optimum specifications updated in 2014 respectively [15, 16, 17]. These analytical goals are derived from BV. The variables used for calculation for Apo A & Apo B were CV% using the respective IQC samples, bias% using the EQAS data and TEa.

There are three levels of analytical goal for imprecision derived from intra - individual BV:
Optimum: CV_A = < 0.25 × $CV_{1,}$ Desirable: CV_A = < 0.50 × $CV_{1,}$ Minimum: CV_A = < 0.75 × CV_1
Where: CV_A = Coefficient of variation (analytical) and CV_I = Coefficient of variation (intra - individual), derived from the intra - individual BV. Coefficient of variation is used to describe the variation of the test. Lower CV denotes a better method performance whereas higher CV implies poorer performance. The degree of precision is usually expressed on the basis of statistical measures of imprecision that is CV. (18)
In our study, from Tables 1 & 2, it is evident that for Apo A1 and Apo B parameters *CV% Analytical* were within acceptable limits as per Desirable & Optimum specifications whereas not acceptable as per Minimum specifications.

There are three levels of analytical goal for bias derived from intra - individual and inter - individual BV:
Optimum: BA=<0.125 (CV2I + CV2G) ½,
Desirable: BA=<0.250 (CV2I + CV2G) ½,
Minimum: BA=<0.375 (CV2I + CV2G) ½

Where: B_A = analytical bias, CV_I = CV of within - subject (intra - individual) BV and CV_G = CV of between—subject (inter - individual) BV. Bias is more difficult to estimate realistically. It is ideal to calculate the bias by using reference method value as "true value. " The most commonly used sources are the ones based on Biological Variation and CLIA guidelines.

External quality assessment schemes (EQAS, also called *proficiency testing*), that is, the percentage deviation of each result with respect to the peer group mean, can be compared with the total error specification shown in this essay to check accuracy which are exactly the same as the values shown in this essay as the "total error" specification, to evaluate the performance of the participating laboratories (19).

In our study, *% BIAS is within acceptable limits as per all 3 specifications.*
The two parameters are conveniently combined as total error allowable (TEa), for which three levels of analytical goal are set:
Optimum: TEa=<1.65 (0.25CVI) +0.125 (CV2I+CV2G) 1/2,
Desirable: TEa=<1.65 (0.50CVI) +0.250 (CV2I+CV2G) 1/2,
Minimum: TEa=<1.65 (0.75CVI) +0.375 (CV2I+CV2G) 1/2

In our study, from Tables 1 & 2, it is also evident that the Total analytical error adopted as bias (%) + 2 CV (%) which is consistent with CLIA recommendations, are within acceptable limits for Apo A1 and Apo B as per Desirable & Optimum specifications whereas not acceptable as per Minimum specifications. Total allowable error biological variability values are the most stringent and perhaps too challenging for analyzing the analytical performance.

The Sigma scale provides guidelines for assay improvement and monitoring. The Six Sigma scale typically runs from zero to six, but a process can actually exceed Six Sigma, if variability is sufficiently low as to decrease the defect rate. Functioning at the 3 - sigma level is regarded as the minimum acceptable level of quality. The six sigma idea asserts an association between the numbers of product defects, wasted operating costs and levels of customer satisfaction. As sigma increases, the consistency, reliability, steadiness and overall performance of the test improves, thereby decreasing the operating costs. For QC procedure, sigma metric analysis is helpful to evaluate the performance and to optimize the protocol for improvement and cost effectiveness. Westgard sigma rules are intended to support laboratory efforts to select statistical QC procedures that are accurate for the specific clinical use and the method performance. The rule is, strive for 6 sigma, >4 sigma is ideal, 6σ –excellent tests - evaluate with 1 QC/day. (alternating levels between days) and 1: 3.5 s rule. • 4 σ - 6 σ - suited for purpose –evaluate with two levels of qc /day, 1: 2.5 s rule • 3 σ - 4 σ –poor performers - use a combination of rules with 2 levels of qc/day.

So Sigma metric analysis is used to measure the performance of a process [20]. In clinical laboratories, assessment of the performance can be done separately for pre analytical, post analytical and analytical phases or as overall laboratory process system. Irrespective of process, sigma metrics covers the five universal steps including define, measure, analyze, improve and control the process. Sigma analysis also identifies errors within the process [21]. In sigma metric analysis, identified errors or defects are considered as poor outcomes which are quantified as DPM or percentage errors. In clinical laboratories, 3 sigma is the

arbitrary value on the sigma scale (ranged from 1 to 6), considered acceptable for process performance. Any laboratory process with the sigma value of 3 is expected to produce 6.7 % clinically unacceptable outcomes [22].

In this study, Six Sigma score was acceptable for Apo A1 under only Optimum quality specification. For Apo B, Six Sigma score was acceptable under both desirable & optimum quality specifications. Although, we could achieve desirable & optimum quality specifications in both analytes, vigorous monitoring & implementing appropriate corrective actions or preventive actions is the key for generating a valid, reliable result for Apo A1 & Apo B. Irrespective of process, sigma metrics covers the five universal steps including define, measure, analyse, improve and control the process. Sigma analysis also identifies errors within the process.

Thus evaluation of the performance of Apo A1 and Apo B helps to minimize the errors and improve process quality. A better analytical quality of these tests can be achieved by setting and implementing evidence - based analytical quality specifications, improving metrological traceability and correcting biases and systematic errors.

5. Conclusion

With the help of ‘‘Westgard sigma rules’’, QC protocol could be customized for better outcome. Each laboratory should monitor the quality control procedures and strategies in a structured manner using sigma metric for improvement of various laboratory processes. Selecting TEa is a key process of laboratory medicine. Results obtained from this study are helpful to minimize the variance and get the optimal quality control procedures for improved quality assurance in Apo A1 & Apo B measurements for the assessment of cardio vascular risk analysis for the patients who are under risk. This study is to evaluate the errors in quality control of analytical phase of laboratory system by sigma metric. For this purpose sigma metric analysis is done for analytes, using the internal and external quality control as quality indicators. Results of sigma metric analysis will be used to identify the gaps and need for modification in the strategy of laboratory quality control procedure.

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