Navigating Lp(a) Measurement Challenges: From Guidelines to Laboratory Perspectives

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Abstract:

This comprehensive review delves into the evolving landscape of Lp(a) measurement standardsand examines the discordance and challenges in cardiovascular risk assessment in the contextof Lp(a). Acknowledging the shifting trends towards universal screening for Lp(a) endorsed by international societies working on risk assessment, management, and prevention of cardiovascular diseases, this review highlights the divergence in recommendations and the lackof consensus on Lp(a) risk thresholds. Ethnic variations in Lp(a) levels and ongoing clinical trial stargeting Lp(a) underscore the urgency of a unified and standardized approach to Lp(a) measurement. This article explores the intricacies of Lp(a) isoform size heterogeneity, availablemeasurement approaches, and the reporting unit challenge for the molecule. Emphasis is placed on collaborative efforts, transparent calibration, and the pivotal role of laboratories in ensuring the precision and reliability of Lp(a) measurements. The discussion encompasses thecall for unified calibration standards, highlighting the need for continuous external quality assessment and transparent reporting in clinical laboratories until a standardized Lp(a) measurement system is established.

Harmonizing Lp(a) Measurement Standards: Bridging Guidelines and Clinical Realities

Updated clinical guidelines and consensus statements/recommendations from various notable international societies working for the prevention, management, and risk assessment of atherosclerotic cardiovascular diseases (ASCVD) have emphasized the significance of evaluating Lp(a) levels in both primary and secondary prevention groups. The European Atherosclerotic Society (EAS)/ European Society of Cardiology (ESC) (2019) and Canadian Cardiovascular Society (CCS) (2021) have advocated universal screening for adults [1]. The landscape of Lp(a) testing has seen a shift towards universal screening, with ongoing debates regarding its benefits and potential implications for cardiovascular disease (CVD) risk assessment and management. The rationale for this approach is based upon, the high epidemiology burden of elevated Lp(a) and accumulating "evidence-based" data from randomized control trials linking Lp(a) to ASCVD. Universal screening is believed to enhance ASCVD risk assessment. Divergent views on universal screening have been reported by the National Lipid Association (NLA) scientific statement. They caution against testing in the general population because of the lack of currently targeted Lp(a)--lowering therapies. Intriguingly, apart from the discordance related to screening of the general population, the professional societal guidelines also lack a generalized consensus on Lp(a) risk thresholds [1].

Given the evidence-based role of Lp(a) as a causal independent CVD risk factor [2], the observed ethnic variations in Lp(a) levels combined with the divergence in recommendations by international societies for screening the population for Lp(a) levels, there is an urgent need fora unified approach to Lp(a) measurement standardization and establishing universally applicable standards for Lp(a) measurement and subsequent generation of Lp(a) risk thresholdsacross geographies.

With multiple ongoing clinical trials exploring Lp(a)-targeting therapies with a ray of hope [3], defining Lp(a) risk thresholds based on a unified standardized approach for Lp(a) measurementwill be of paramount importance for effective risk and clinical management. In this direction, the concerted efforts by the International Federation of Clinical Chemistry (IFCC) and laboratories shall play a crucial role in standardizing and harmonizing Lp(a) measurements, thereby ensuring consistency across diverse populations. Addressing Lp(a) measurementissues, transparent calibration, and adopting reliable methodologies are imperative for an accurate risk assessment. Furthermore, collaborative efforts among laboratories, clinicians, and researchers are essential for establishing comprehensive guidelines that account for ethnic variations, advancing precision in Lp(a) risk evaluation, and therapeutic interventions.

The following discussion aims to provide insights into the challenges, advancements, and Collaborative strategies that can contribute to the harmonization of Lp(a) measurement standards, enhance clinical interpretation, and facilitate comprehensive insights into its role incardiovascular health.

Navigating challenges in Lp(a) measurement

As the landscape of cardiovascular risk assessment evolves, the identification of Lp(a) as a distinct contributor to cardiovascular health and its incorporation into the risk prediction framework highlights the critical role of accurate measurements in assessing and guiding therapeutic interventions, signifying a paradigm shift in the nuanced comprehension and management of CVD risk [2].

Peculiarities of Lp(a) structure: Implications for Isoform Heterogeneity and Challenges in Measurement"

While navigating the challenges associated with Lp(a) measurement, it was observed that this enigmatic molecule has inherent structural peculiarities that cause 'isoform heterogeneity' and that leads to a major analytical challenge. Lp(a) has a unique apolipoprotein structure consisting of 11 types of Kringle sequences (Kringle IV type 1 to type 10; Kringle V) and a protease domain. Kringle IV type 2 shows sequence polymorphism with a variable number of repeated copies (ranging from 3 to >40) [4], which creates diverse apo(a) isoform landscapes and leads to molecular weights ranging from approximately 250 to 800 kDa [5]. Notably, there is an inverse relationship between the isoform size of Lp(a) and its plasma concentration. The repetitive Kringle IV2 (KIV2) structure and the high homology between these repetitive units pose a substantial hurdle for the precise measurement of the encoded protein. This isoform heterogeneity introduces 'measurement bias' that complicates the precise quantification [6,7] and this potential 'measurement bias' further influences the measurement outcomes.

Precise Quantification of Lp(a): What are the available approaches to measuring Lp(a)?

We have different analytical assays available to measure Lp(a) concentration in serum: isoform-sensitive, isoform-insensitive, and newer assays based on mass spectrometry. The widely available, commercially utilized, immunoturbidometric or immunonephelometric assays use antibodies raised in animals that are polyclonal in nature and cross-react with variable epitopes of apo(a). This polyclonality raises concerns about potential "measurement bias" with consequences.

The bias manifests as smaller isoforms that are linked to elevated levels being underestimated, whereas large isoforms with numerous KIV repeats associated with lower levelsare overestimated [8]. Furthermore, this bias affects theassociation between Lp(a) and ASCVD risk [9] and the misclassification of patients with Lp(a) levels close to predefined cut-off points [9].

An alternative approach for Lp(a) measurement available is ELISA, using a monoclonal antibody directed against the apoB of Lp(a). This assay is insensitive to the apo(a) isoform size heterogeneity [6]. The limited adoption of this method may be attributed to overlooking the LDL-unbound-'free' apo(a) (approx. 5%) [10,11], with uncertain implications for apo(a) atherogenicity [10].

A newer approach that is well-suited for high-throughput applications, marking a significant stride in precision and efficiency in Lp(a) analysis, is a mass spectrometry-based approach [12]. Unlike traditional immunochemical assays, this method identified unique peptide fragments of apo(a) that were absent in the KIV2 domain, mitigating issues of isoform size bias. However, cost concerns currently limit its application in routine clinical practice.

Prevalence of assays reporting Lp(a) values as mass concentration (mg/dl) vs. particle concentration (nmol/L): The reporting unit challenge

Recently, the discontinuation of the use of mg/dl units for Lp(a) reporting has been recommended [13], and it has been observed that the measurement of Lp(a) in particle concentration units (nmol/L) is being increasingly adopted. Numerous high-throughput platforms employing methods such as immunoturbidimetry or immunonephelometry now report Lp(a) measurements in nmol/L.

The primary question here is why we need Lp(a) concentration to be reported as nmol/L, and secondly, can mg/dl be converted to nmol/l and vice versa for Lp(a)?

Expressing Lp(a) as a mass concentration (mg/dL) introduces inherent bias because a given mass represents fewer particles for large isoforms and more particles for small isoforms. Furthermore, because Lp(a) isoforms have different molecular weights, direct conversion is not possible, unlike that of other lipids and lipoproteins [9]. Furthermore, commercial assays that incorporate five isoform sizes as calibrators are standardized against the WHO/IFCC reference material, which is reported in nmol/L units to effectively minimize isoform size bias [7].

From a technical standpoint, most assays in clinical practice utilize polyclonal antibodies against Lp(a) and are inherently isoform-sensitive [7,14,15]. However, some assay manufacturers have claimed that their assays demonstrate isoform insensitivity by making direct comparisons with isoform-insensitive assays [12]. This approach is good, but its limitations include inadequate representation of certain apo(a) isoform groups (smaller ones) owing to the limited sample pool. Additionally, some assay manufacturers are claiming a conversion factor between 2.0 and 2.5 from mg/dl to nmol/l without providing reasons behind [16]. This conversion with a fixed factor of 2.5 leads to overestimation for larger isoforms and underestimation for smaller isoforms. Furthermore, some assay manufacturers offer both mass and molar assays for Lp(a), despite using similar types of polyclonal antibodies, the same calibrator, and the same measurement system. Given the structural heterogeneity of Lp(a), thisis highly unlikely.

Enhancing Lp(a) Measurement Accuracy: Multipoint Calibrators, Transparencyin Calibration, and Certification Processes

Overcoming the above-mentioned challenges is pivotal for refining Lp(a) measurement methodologies and ensuring alignment with their true physiological significance. Researchers and laboratories are actively pursuing strategies to address this complexity, ultimately helpingto enhance the reliability and clinical relevance of Lp(a) measurements in cardiovascular healthassessments.

To optimally minimize the major obstacle for Lp(a) measurement i.e., isoform size bias, the useof multipoint calibrators (5- 5-point calibrator) is suggested. The multipoint calibrator shouldcover the measured concentration range and each calibrator should be independent and containa suitable distribution of apo(a) isoforms traceable to the WHO/IFCC reference material [7]. Additionally, the assay manufacturers must transparently disclose crucial information regardingthe apo(a) isoform size associated with each calibration and specify that the multipoint calibrator is a dilution of a single calibrator or consists of various calibrators with different apo(a) isoform sizes. A clear and comprehensive disclosure of these aspects will ensure the integrity and reliability of the Lp(a) measurements across diverse calibration points [16]. Furthermore, the Northwest Lipid Metabolism and Diabetes Research Laboratory (NLMDRL) at the University of Washington, Seattle, offers a certification process for assessing the performance of various assays. This involved comparing the Lp(a) values obtained through thecertification process with those derived from the monoclonal antibody-based ELISA method [7].

Laboratory Perspectives on Lp(a): Investigating the Enigma and Advancing Clinical Understanding

Given the role of Lp(a) as an independent causal risk factor for CVD, Lp (a) measurements needto be standardized. This involves addressing the technical challenges, implementing unified calibration standards, and ensuring continuous External Quality Assessment Schemes (EQAS). Concerted efforts by laboratories and researchers should aim to enhance measurement accuracy, resolve technical intricacies, and promote uniform reporting practices. Such initiativeswill strengthen the reliability of Lp(a) assessments, offering a foundation for precise clinical interpretation and facilitating comprehensive insights into their role in cardiovascular health.

Call for Unified Calibration Standards

The demand for Lp(a) measurement is expected to surge, particularly if ongoing trials of Lp(a)-lowering drugs yield positive results. High-throughput methods, often based on immunoturbidimetric and immunonephelometric techniques, are essential to meet this demand. A recent study compared the performance of six widely utilized commercially available assays that use five-point calibrators provided by manufacturers on a sample size of 144 serumsamples to an assay that claims to be isoform-insensitive (Denka Reagents). The overall bias between the assays ranged from -5 to +15 mg/dl. This suggests that all assay manufacturers should use a particular set of calibrators [17].

Furthermore, along with other scientific contributors [12], the IFCC Working Group for Standardization of Apo lipoproteins by Mass Spectrometry [18] is diligently involved in crafting advanced reference materials using contemporary methodologies. These materials are universally available and can serve as calibration standards for assay manufacturers. Leveragingthe inherent robustness of mass spectrometry technology facilitates the precise and reproducible quantification of proteins at the molecular level. This positions mass spectrometry as the benchmark reference method for clinical assay comparisons, particularly in molar units.The anticipated accessibility of these newly calibrated reference materials will present an opportunity for assay manufacturers to enhance the calibration precision of their methods.

The Crucial Role of Continuous External Quality Assessment in Lp(a) measurement

Clinical laboratories must systematically undergo external quality assessment to promptly identify and address disruptions introduced throughout the chain, from assay production to clinical application.

This ongoing evaluation is indispensable for upholding the integrity and reliability of laboratory processes, facilitating the early detection and effective mitigation of potential disturbances. Continuous participation in external quality assessment programs serves as a robust mechanism to ensure the precision and consistency of laboratory procedures,thus contributing significantly to the overall reliability of clinical practice. External quality assurance programs should distribute samples with known apo(a) isoform compositions and Lp(a) values assigned by a validated method independent of apo(a) size polymorphism and withcalibration traceable to the WHO/IFCC SRM-2B reference material [7].

Furthermore, external quality assurance samples should encompass a clinically relevant range, particularly within the management threshold range of 90-200 nmol/L. This comprehensive approach ensures thorough assessment and validation of laboratory performance across key parameters [7,16].

Transparent Reporting in Clinical Laboratories

Until a unified standardized system for Lp(a) measurements has been generated, clinical laboratories must transparently report not only the measured Lp(a) concentration but also specify the assay and calibrators employed. This is crucial for accurate clinical interpretation, particularly for serial measurements conducted across different laboratories in some cases. The laboratory must indicate the reported unit, as confusion often arises from unit discrepancies, impacting daily clinical counseling. This straightforward disclosure ensures consistency, eliminates ambiguity, and enhances the reliability of the Lp(a) concentration data in clinical settings [16].

Conclusion

The complex landscape of Lp(a) measurement requires a concerted effort to standardize and harmonize practices for precise clinical interpretation. The divergence in recommendations from international societies underscores the critical need for a unified approach to Lp(a) measurement standardization. As clinical guidelines advocate universal screening and ongoingtrials exploring Lp(a)-targeting therapies, establishing universally applicable standards and defining risk thresholds are paramount.

Laboratories play a pivotal role in this endeavor by addressing challenges, such as isoform heterogeneity and reporting units. Multipoint calibrators, transparent calibration, and certification processes are crucial for accurate quantifications. Collaboration among laboratories, clinicians, and researchers is essential for comprehensive guidelines that consider ethnic variations and advance precision in Lp(a) risk evaluation. Continuous external quality assessment further ensured the reliability of laboratory procedures.

As we navigate the complexities of Lp(a) measurement, transparent reporting practices have become a temporary solution, enhancing consistency across diverse laboratories. Until a unifiedstandard is established, these initiatives collectively contribute to bridging guidelines with clinical realities, ultimately advancing our understanding of Lp(a) in cardiovascular health.

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