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Comparison of lipoprotein (a) serum concentrations measured by six commercially available immunoassays

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HIGHLIGHTS

- Lipoprotein (a) is an established risk factor for cardiovascular disease
- The comparison between commercially available assays revealed substantial differences
- Further efforts to harmonize assays for lipoprotein(a) are needed

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ABSTRACT

Background and aims: Lipoprotein (a) [Lp(a)] is an established causal risk factor for cardiovascular disease (CVD), independently of low-density lipoproteins (LDL) and other risk factors. The recognition of Lp(a) as an atherogenic molecule has raised the demand for reliable quantification methods in the clinical laboratory. The aim of this work is to compare commercial immunochemical assays.

Methods: We measured Lp(a) serum concentrations using six different assays, providing Lp(a) in mg/dl (Denka Seiken, Abbott Quantia, Beckman, Diasys 21FS, and Siemens N Latex) or in nmol/l (Roche TinaQuant, Diasys 21 FS) in 144 serum samples covering the clinically relevant range of Lp(a) concentrations. All assays relied on five-point calibrations using calibrators provided by the manufacturers. Apolipoprotein(a) phenotyping was performed by sodium dodecyl sulfate-agarose gel electrophoresis (SDS-agarose) followed by immunoblotting.

Results: Most bivariate correlation coefficients were greater than 0.90. Compared to an established IFCC-proposed reference material, the results of the different assays diverged from the target values (43.3 mg/dl or 96.6 nmol/l) by −8% (Siemens N Latex) and +22% (Abbott Quantia). Stratification of the samples into five groups with increasing Lp(a) concentrations and difference plots showed that the differences among assays were concentration-dependent. Some assays overestimated Lp(a) at high concentrations compared to the Denka Seiken assay.

Conclusions: Current commercial immunological assays for measuring Lp(a) concentrations are differently calibrated. Their biases differ significantly across the clinically relevant concentration range in a non-linear manner. This is not conclusively explained by apolipoprotein (a) phenotypes. Further international efforts to harmonize assays for Lp(a) are needed.

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1. Introduction

Lipoprotein (a) (Lp(a)) is a causal risk factor for the development of cardiovascular disease (CVD), independent of low-density lipoproteins (LDL) and other risk factors [1–3]. The risk of developing coronary artery diseases (CAD) in Caucasians is more than two times higher at elevated Lp(a) [4,5]. Causality of the relationship between Lp(a) plasma concentration and CVD was first reported in 1992 [6] using the algorithm that was denominated in subsequent studies "Mendelian Randomization" [7]. Plasma Lp(a) concentrations vary from less than 0.1 mg/dl to more than 300 mg/dl and their frequency distribution is strongly skewed to the right in the white population. A cut-off concentration of 30 mg/dl has originally been defined above which the risk of myocardial infarction increases [8]. A meta-analysis of prospective studies enrolling a total of more than 126.000 individuals [5] found incidence rates of coronary heart disease in the top and bottom tertiles of Lp(a) of 5.6 (95% CI, 5.4–5.9) and 4.4 (95% CI, 4.2–4.6) per 1000 person-years. This meta-analysis also shows that the relationship between Lp(a) and CHD might not be strictly continuous, in that significant increases of risk were only seen when Lp(a) concentrations exceed 30 mg/dl and graded increases in risk may occur above this threshold. A less rigorous cut-off of 50 mg/dl has also been suggested [1].

Lp(a) is an LDL-like lipoprotein to which apolipoprotein (a) [apo(a)] is linked through a disulfide bridge. Apo(a) consists of repetitive domains, so called kringles (K), which are highly homologous to kringle four (K-IV) of plasminogen [9]. Apo(a) in addition contains one copy of a plasminogen K-V-like domain and an enzymatically inactive protease. In humans, more than 30 genetically determined apo(a) size isoforms (phenotypes) exist; their sizes are inversely correlated with circulating Lp(a) concentrations accounting for approximately 50% of the inter-individual concentration differences [10], yet numerous additional polymorphisms and mutations with varying functionality have been described [11]. The size isoforms differ by varying numbers of identical copies of K-IV type 2; the smallest apo(a) contains 11, the largest one more than 54 ones [12]. The sequences of the remaining K-IV motifs differ from K-IV type 2 and among themselves but share a high degree of homology. As a consequence of the variations in the number of KIV-type repeats, the molecular weights of the apo(a) phenotypes (which also includes a considerable degree of glycosylation) range from approx. 300 to more than 800 kD [13].

The recognition of Lp(a) as a causal atherogenic molecule has raised the demand for reliable methods of quantification. Lp(a) has mostly been measured with immunochemical methods relying on the detection of its apo(a) moiety. One of the problems in the quantification of Lp(a) arises from the size polymorphism of apo(a) [14]. While the epitopes recognized by the majority of antibodies in commercial assays are unknown, they are likely to bind to epitopes residing both on the repetitive and non-repetitive K domains. Because the K-IV domains share a high degree of homology, apo(a) is expected to contain many 'repetitive' epitopes, the number of which varies in parallel to the number of repetitive K-IV type 2 domains in a given individual. Therefore, depending on the assay type and antibody specificity, the size polymorphism of apo(a) may impact the results. For instance, the molar concentration of Lp(a) may be underestimated in individuals with small isoforms and high concentrations, while it may be overestimated in individuals with large isoforms and low concentrations when one uses a single reference standard [15]. Further methodical issues are: (i) Lp(a) particles additionally vary in molar mass and hydrated density, independently of the apo(a) size polymorphism; (ii) purification of Lp(a) for primary standards is difficult and purified Lp(a) is unstable; and (iii) Lp(a) forms mixed aggregates with LDL that are not always fully dissociable (for a comprehensive review covering this topic, see Lippi et al. [16]).

To deal with these problems, a Lp(a) standardization working group supported by the WHO and the IFCC was established [17,18]. This

Table 1
Immunological assays used for the measurement of Lp(a).

Assay	Unit	Calibrators (n)	Method	Analyser	Site/laboratory	Detection antibody	Reference material
Denka Seiken	mg/dl	5[e]	Immunturbidimetry	Olympus AU640	CIMCL, Graz[a]	Anti-Lp(a) (rabbit), polyclonal	Internal reference material not specified
Abbott Quantia	mg/dl	5[e]	Immunturbidimetry	Abbott Architect c16000	Lab Med, Linz[b]	Anti-Lp(a) (rabbit), polyclonal	Internal reference material not specified
Beckman	mg/dl	5[e]	Immunturbidimetry	Olympus AU640	CIMCL, Graz[a]	Anti-Lp(a) (rabbit), polyclonal	Internal reference material not specified
DiaSys 21 FS a[c]	mg/dl	5[e]	Immunturbidimetry	Olympus AU640	CIMCL, Graz[a]	Anti-Lp(a) (rabbit), polyclonal	SRM2B
DiaSys 21 FS b[c]	nmol/l	5[e]	Immunturbidimetry	Olympus AU640	CIMCL, Graz[a]	Anti-Lp(a) (rabbit), polyclonal	SRM2B
Roche TinaQuant	nmol/l	5[e]	Immunturbidimetry	Roche Cobas c501	Lab Med, Linz[b]	Anti-Lp(a) (rabbit), polyclonal	SRM2B
Roche TinaQuant converted[d]	mg/dl	5	Calculation (nmol/l * 0.4167)				
Siemens N Latex	mg/dl	5[f]	Nephelometry	BN II Nephelometer	CIMCL, Graz[a]	Anti-Lp(a) (rabbit), polyclonal	Internal reference material not specified

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[c] Diasys 21 FS a and Diasys 21 FS b are independent assays, each using five calibrators provided by the manufacturer, the two assays therefore provide different results.
[d] Results from the Roche TinaQant, which are in nmol/l, were converted into mg/dl using Lp(a) (mg/dl) = Lp(a) (nmol/l) * 0,4167 as suggested by the manufacturer of the assay.
[e] Five different calibrator pools.
[f] Serial dilution of one calibrator pool.

Table 2
Mean Lp(a) concentrations obtained in two control samples analyzed on six consecutive working days with commercial assays.

Assay	Control 1				Control 2			
	Mean	SD	CV	Difference (%)[a]	Mean	SD	CV	Difference (%)[a]
Denka Seiken (mg/dl)	103	3	2.6	reference	13	1	4.7	reference
Abbott Quantia (mg/dl)	126	2	1.6	+22	17	0,4	1.8	+31
Beckman (mg/dl)	87	11	13.0	−16	11	1	12	−15
DiaSys 21 FS a[b] (mg/dl)	121	4	3.5	+17	10	0.1	1.1	−23
DiaSys 21 FS b[b] (nmol/l)	237	10	4.2		22	2	7.2	
Roche TinaQuant (nmol/l)	260	6	2.3		23	0.5	2.2	
Roche TinaQant converted[c] (mg/dl)	109	2.5	2.3	+6	10	0.2	2.2	−23
Siemens N Latex (mg/dl)	107	5	4.7	+4	15	2	13	+15

[a] The entries contain means with standard deviations of two control samples (top rows) analyzed on six consecutive working days and the relative differences of the respective mean value from the mean value of the Denka Seiken assay. For assays providing Lp(a) in nmol/l we did not calculate the differences.
[b] Diasys 21 FS a and Diasys 21 FS b are independent assays, each using five calibrators provided by the manufacturer, the two assays therefore provide different results.
[c] Results from the Roche TinaQant, which are in nmol/l, were converted into mg/dl using Lp(a) (mg/dl) = Lp(a) (nmol/l) * 0.4167 as suggested by the manufacturer of the assay.

Table 3
Mean Lp(a) concentrations of the IFCC-PRM-1 reference material with an assumed target value of 43.3 mg/dl or 96.6 nmol/l, observed on two different working days.

Assay	Mean of 2 measurements[a]	Target value	Difference (%)
Denka Seiken (mg/dl)	48.5	43.3	+12
Abbott Quantia (mg/dl)	52.7	43.3	+22
Beckman (mg/dl)	51.5	43.3	+19
DiaSys 21 FS a[b] (mg/dl)	46.0	43.3	+6
DiaSys 21 FS b[b] (nmol/l)	104.7	96.6	+8
Roche TinaQuant (nmol/l)	109.4	96.6	+13
Roche TinaQant converted[c] (mg/dl)	45.6	43.3	+5
Siemens N Latex (mg/dl)	39.7	43.3	−8

[a] The PRM-1 reference material was included in single measurement on days 1 and 4 of our study.
[b] Diasys 21 FS a and Diasys 21 FS b are independent assays, each using five calibrators provided by the manufacturer, the two assays therefore provide different results.
[c] Results from the Roche TinaQant, which are in nmol/l, were converted into mg/dl using Lp(a) (mg/dl) = Lp(a) (nmol/l) * 0.4167 as suggested by the manufacturer of the assay.

group found a large between-method variation mainly due to the lack of a common reference material for Lp(a). Although most manufacturers mention in their protocol that their assays were standardized with the IFCC-proposed reference material (PRM)-2 [17], vast differences of Lp (a) concentrations reported by different laboratories are regularly encountered in clinical practice. We therefore sought to systematically compare currently used assays and methods in clinical laboratories.

2. Materials and methods

2.1. Serum samples

We selectively collected 144 fasting serum samples from patients attending the Medical University of Graz, in which Lp(a) measurements were requested for clinical reasons and which covered the entire, clinically relevant range of Lp(a) concentrations. Serum was recovered by centrifugation, aliquoted and stored at −20° C at maximum for 90 days. According to the Siemens N Latex assay, which is currently used in our routine laboratory, the Lp(a) concentrations in our samples were distributed as follows: 2.5–10 mg/dl, n = 15; 11–20 mg/dl, n = 15; 21–30 mg/dl, n = 15; 31–40 mg/dl, n = 15; 41–50 mg/dl, n = 15; 51–60 mg/dl, n = 17; 61–70 mg/dl, n = 14; 71–90 mg/dl, n = 15; 91–110 mg/dl, n = 11; > 110 mg/dl, n = 13. The study was approved by the ethics review committee of the Medial University of Graz (ek 27-305ex 14/15).

2.2. Determination of the Lp(a) concentration

We measured Lp(a) with five immunoturbidimetric assays and one immunonephelometric assay using the conditions shown in Table 1. All assays were conducted as single values in aliquots frozen and thawed once. Because the Abbott Quantia and the Roche Tina Quant assays were not available at the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Graz, we provided frozen aliquots of the samples to the Department of Laboratory Medicine, Konventhospital Barmherzige Brüder, Linz, for running the Abbott Quantia and the Roche TinaQuant assays. Samples were shipped on dry ice.

The immunoturbidimetric assays used five-point calibration curves constructed with five lyophilized standards which were reconstituted separately. The immunonephelometric assay (Siemens N Latex) used a five-point calibration curve generated by serial dilutions of a Lp(a) standard, the dilutions were automatically prepared by the analyser. Diasys 21 FS a and Diasys 21 FS b provide Lp(a) calibrator values in mg/dl and in nmol/l, respectively. Both use independent calibrators provided by the manufacturer, and we therefore evaluated the two assays independently. The Roche TinaQuant assay measures Lp(a) in nmol/l; for comparison purposes, we converted these values into mg/dl by multiplication with the factor 0.4167 recommended by the manufacturer and report the results as Roche TinaQuant converted.

The analyses were conducted on six consecutive working days in each of the participating laboratories and the samples were allocated randomly to these days: day 1, n = 23; day 2, n = 25, day 3, 25; day 4, n = 23; day 5, n = 24; day 6, n = 24. Thereby, we made sure, that samples across the entire range of Lp(a) concentrations were examined on each day. Into each run, we included two control samples (low and high level) the position of which within the individual batches was blinded to the technicians performing the analyses. We also included as a reference material the PRM-1 originally prepared by the IFCC working group. The reference material was prepared from purified Lp (a), consisting of 24 (predominant band) kringle 4 domains [17]. A target value of 96.6 nmol/l or 43.3 mg/dl was adopted for this material. PRM-1 was stored since 1999 in lyophilized form at minus 70 °C.The PRM-1 reference samples were measured at a blinded position on days 1 and 4.

2.3. Determination of apo(a) phenotypes

Apo(a) phenotyping was performed by sodium dodecyl sulfate-agarose gel electrophoresis (SDS-agarose) of serum under reducing

Table 4
Regression according to Passing and Bablock and correlation coefficients according to Spearman and Pearson of immunological assays for measuring Lp(a).

	Denka Seiken (mg/dl)	Abbott Quantia (mg/dl)	Beck-man (mg/dl)	DiaSys 21 FS a (mg/dl)	DiaSys 21 FS b (nmol/l)	Roche TinaQuant (nmol/l)	Roche (mg/dl)	Siemens N Latex (mg/dl)
Denka Seiken (mg/dl)		0.958 0.922	0.935 0.966	0.956 0.990	0.949 0.990	0.943 0.995	0.943 0.995	0.907 0.948
Abbott Quantia (mg/dl)	1.31 0.59		0.933 0.959	0.985 0.984	0.977 0.984	0.985 0.995	0.985 0.996	0.952 0.965
Beck-man (mg/dl)	0.926 -0.632	0.699 -0.949		0.938 0.959	0.928 0.961	0.931 0.963	0.931 0.963	0.854 0.900
DiaSys 21 FS a[b] (mg/dl)	1.080 -4.762	0.836 -4.339	1.225 -2.962		0.995 0.995	0.987 0.987	0.987 0.987	0.938 0.945
DiaSys 21 FS b[b] (nmol/l)	2.20 -10.24	1.71 -10.65	2.52 -7.08	2.05 -1.69		0.986 0.987	0.986 0.987	0.925 0.936
Roche TinaQuant (nmol/l)	2.44 -8.94	1.92 -10.14	2.78 7.21	2.28 0.11	1.12 0.22			0.942 0.957
Roche TinaQant converted[c] (mg/dl)	1.01 -3.58	0.80 -4.16	1.14 -2.53	0.95 0.15	0.47 0.27			0.942 0.957
Siemens N Latex (mg/dl)	1.29 0.68	0.96 0.04	1.41 0.19	1.11 6.39	0.54 7.86	0.48 6.18	1.19 5.29	

[a] The fields above the diagonal contain non-parametric Spearman correlation coefficients (top) and correlation coefficients according to Pearson (bottom). The fields below the diagonal contain the slopes (top) and the intercepts (bottom) of the regression lines, all calculated according to Passing and Bablock [21]. Regression analyses were calculated using assays in columns as independent variable (X) and the methods in the rows as dependent variable (Y). The dark grey fields contain assays producing results in different units. In these cases, the slopes necessarily depart from unity.
[b] Diasys 21 FS a and Diasys 21 FS b are independent assays, each using five calibrators provided by the manufacturer, the two assays therefore provide differenr results.
[c] Results from the Roche TinaQant, which are in nmol/l, were converted into mg/dl using Lp(a) (mg/dl) = Lp(a) (nmol/l) * 0.4167 as suggested by the manufacturer of the assay.

conditions as previously described [19]. All samples were diluted with phosphate buffer to a standardized Lp(a) concentration of approximately 30 ng/µl. Samples with an Lp(a) concentration < 15 ng/µl were not diluted, but a higher volume of sample was applied on the gel to obtain an equal apo(a) mass. A mixture of five plasma samples expressing only one apo(a) isoform each (13, 19, 23, 27, and 35 KIV repeats, determined by pulsed-field gel electrophoresis) was applied as apo(a) isoform size standard. SDS-agarose electrophoresis was followed by immunoblotting using the monoclonal anti-apo(a) antibody 1A2 [20] for detection of apo(a) phenotypes. Samples with at least one isoform with less than 23 KIV repeats were considered as low molecular weight (LMW) apo(a); high-molecular weight (HMW) isoforms contained 23 or more KIV repeats.

2.4. Statistical analysis

We used least squares regression, Pearson and Spearman correlation coefficients and the non-parametric method of Passing and Bablock [21] implemented in the tool Analyze-it for Microsoft Excel for bivariate comparisons (https://analyse-it.com). Day to day imprecision was expressed as the coefficient of variation of the two control samples included in each run.

3. Results

3.1. Imprecision

Table 2 shows the means and standard deviations of Lp(a) concentrations obtained for the two control samples analyzed on six consecutive working days. Day-to-day imprecision was in general low, with the exception of the Beckman assay which revealed coefficients of variation of 12 and 13% in the low- and the high-level control sample, respectively.

3.2. Accuracy

In the high-level control sample (Table 2), mean Lp(a) concentrations decreased in the following order (assays with mg/dl): Abbott Quantia > Diasys 21 FS a > Roche TinaQuant converted > Siemens N Latex > Denka Seiken > Beckman. The mean concentration obtained with Roche TinaQuant (nmol/l) was higher than that of the DiaSys 21 FS b (nmol/l). For the low-level control the ranking was different: Abbott Quantia > Siemens N Latex > Denka Seiken, > Beckman > Diasys 21 FS a > Roche Tina Quant converted.

We also measured Lp(a) in the PRM-1 reference material (Table 3). The results suggested that the assays were differently calibrated. The deviations from the target values (96.6 nmol/l or 43.3 mg/dl) ranged from −8% (Siemens N Latex) to +22% (Abbott Quantia). The Denka Seiken assay is only slightly affected by the apo(a) size variation [14]. For this reason, we compared all further analyses using this assay as provisional reference.

3.3. Assay comparison

The bivariate correlation coefficients for pairwise comparisons were greater than 0.90 throughout (Table 4, upper diagonal), with the

Table 5
Mean ± standard deviations, medians and interquartile ranges of Lp(a) concentrations obtained with commercial assays of 144 samples.[a]

Assay/concentration range (mg/dl)	0–175	≤19.9	20–39.9	40–59.9	60–79.9	80–175
N =	144	29	30	32	21	32
Denka Seiken (mg/dl)	43 ± 29	8 ± 4	22 ± 8	46 ± 14	56 ± 12	82 ± 19
	40 (16–63)	8 (4–11)	20 (16–26)	43 (37–52)	55 (49–60)	82 (66–101)
Abbott Quantia (mg/dl)	57 ± 41	12 ± 5	29 ± 7	56 ± 15	74 ± 16	116 ± 31
	48 (23–80)	12 (7–16)	27 (24–32)	51 (44–66)	73 (63–80)	110 (101–130)
	+33/+20	+50/+49	+29/+37	+21/+73	+31/+32	+41/+35
Beckman (mg/dl)	38 ± 29	8 ± 3	17 ± 7	41 ± 15	52 ± 14	72 ± 28
	34 (14–53)	8 (5–10)	16 (13–19)	37 (29–51)	56 (42–63)	70 (50–92)
	−12/−15	+1/−1	−24/−21	−12/−16	−8/+1	−12/−14
DiaSys 21 FS a[b] (mg/dl)	44 ± 36	6 ± 3	17 ± 9	43 ± 15	56 ± 12	92 ± 31
	38 (12–62)	6 (4–8)	15 (12–19)	42 (35–51)	56 (48–60)	86 (69–110)
	+2/−5	−40/−23	−40/−26	−28/−4	−23/+2	−14/+6
DiaSys 21 FS b[b] (nmol/l)	89 ± 73	12 ± 7	35 ± 17	86 ± 34	112 ± 28	184 ± 66
	78 (26–131)	11 (6–17)	31 (26–37)	79 (65–104)	111 (90–118)	176 (136–222)
	–	–	–	–	–	–
Roche TinaQuant (nmol/l)	100 ± 83	13 ± 6	41 ± 17	101 ± 32	129 ± 27	212 ± 80
	87 (29–146)	13 (7–18)	36 (29–48)	93 (78–124)	131 (113–143)	192 (165–230)
	–	–	–	–	–	–
Roche TinaQant converted[c] (mg/dl)	42 ± 35	6 ± 3	17 ± 7	42 ± 13	54 ± 11	88 ± 33
	36 (16–57)	6 (3–8)	15 (12–20)	39 (32–52)	55 (47–59)	80 (69–96)
	−3/−10	−28/−29	−22/−25	−9/−11	−4/−1	+8/−2
Siemens N Latex (mg/dl)	54 ± 38	10 ± 5	30 ± 6	50 ± 6	68 ± 6	111 ± 26
	49 (25–75)	11 (5–13)	30 (25–36)	51 (45–55)	68 (62–71)	104 (91–128)
	+26/+23	+29/+42	+35/+49	+9/+17	+21/+23	+36/+27

[a] The entries contain means with standard deviations (top rows), medians with interquartile ranges (medium rows) and the relative differences of the median and the mean values in percent compared to the median and mean values of the Denka Seiken assay in the complete set of samples and in groups of samples with different Lp(a) concentrations. The stratification of samples according to Lp(a) was conducted using the results of the Siemens N Latex assay at the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz. Relative differences between Roche TinaQuant (nmol/l) and DiaSys 21 FS b (nmol/l) and Denka Seiken (mg/dl) were not calculated.

[b] Diasys 21 FS a and Diasys 21 FS b are independent assays, each using five calibrators provided by the manufacturer, the two assays therefore provide different results.

[c] Results from the Roche TinaQant, which are in nmol/l, were converted into mg/dl using Lp(a) (mg/dl) = Lp(a) (nmol/l) * 0.4167 as suggested by the manufacturer of the assay.

exception of the correlation between the Siemens N Latex and the Beckman assay (r according to Spearman = 0.854, Table 4). The slopes of the regression lines of the Denka Seiken assay ranged between 0.93 and 1.31 by considering only assays providing Lp(a) in mg/dl (Table 4, lower diagonal, left column); the slopes of the regression lines compared to the Denka Seiken assay decreased in the following order: Abbott Quantia > Siemens N Latex > DiaSys 21 FS a > Roche TinaQuant converted > Beckman. The same ranking was found when we considered the slopes of least squares regression lines (Table 4).

Comparison of the mean and median values in our 144 samples also reflected the different calibrations of the assays. Overall the mean values were in the following order: Abbott Quantia > Siemens N Latex > Diasys 21 FS a > Denka Seiken > Roche TinaQuant converted > Beckman (Table 5).

Stratification of the results into five groups with increasing Lp(a) concentrations (obtained originally with the Siemens N Latex assay) indicated that the differences between the assays were concentration-dependent (Table 5). We therefore generated difference plots in comparison to the Denka Seiken assay (Fig. 1A–E). The Abbott Quantia assay positively deviated from Denka Seiken assay, less so at low, but markedly at high concentrations. Compared to the Denka Seiken assay, the Beckman assay slightly underestimated Lp(a) concentrations, whereby the differences scattered randomly and widely at concentrations above 30 mg/dl. The DiaSys 21 FS assay coincided well with the Denka Seiken assay at concentrations up to 80 mg/dl; beyond this value it clearly overestimated Lp(a). The same applied to the Roche TinaQuant converted. The Siemens N Latex assay underestimated Lp(a) below 20 mg/dl, but overestimated Lp(a) above this value in comparison to the Denka Seiken assay. The DiaSys 21 FS b (nmol/l) assay underestimated Lp(a) in relation to the Roche TinaQuant assay (nmol/l) which became particularly apparent at concentrations above 50 nmol/l (Fig. 1F).

3.4. Apo(a) phenotypes

We examined whether the relative differences between the assays were related to the smallest of the apo(a) isoform(s) expressed in each sample and grouped them in LMW (less or equal than 22 K-IV repeats) or HMW (more than 22 K-IV repeats) apo(a) phenotypes (Table 6, Fig. 1A–F). Table 6 shows that in some cases the relative differences seen in samples with HMW apo(a) were identical to the relative difference in samples with LMW apo(a), in other cases biases were different (e.g. Diasys 21FS or RocheTinaQant converted versus Denka Seiken).

As expected, the difference plots showed an overlap of samples with HMW apo (a) and LMW apo(a) in the concentration range between 40 and 70 mg/dl (Fig. 1). We could, however, not find discernible shifts of the differences within this concentration range (which would have been anticipated if the differences were related to apo(a) size). An exception was the difference plot of the Siemens N Latex versus the Senka Daiken assay (Fig. 1E) in which the HMW apo(a) samples had a stronger positive bias than the LMW apo(a) samples.

We also generated difference plots with relative rather than absolute differences again accounting for HMW and LMW apo(a) (Supplementary Fig. 1). Only at very low Lp(a) concentrations did the Abbott Quantia (Supplementary Fig. 1A), the Beckman (Supplementary Fig. 1B), the Diaysys 21 FS a (Supplementary Fig. 1C), and the Roche TinaQant converted (Supplementary Fig. 1D) show positive proportional bias in comparison to higher concentrations. This may, however, be of limited practical significance, because it applies to Lp(a) concentrations far below clinical decision limits. Finally and deviating from the other methods, the Siemens N Latex assay in tendency showed a positive bias across all HMW apo(a) samples (Supplementary Table 1E).

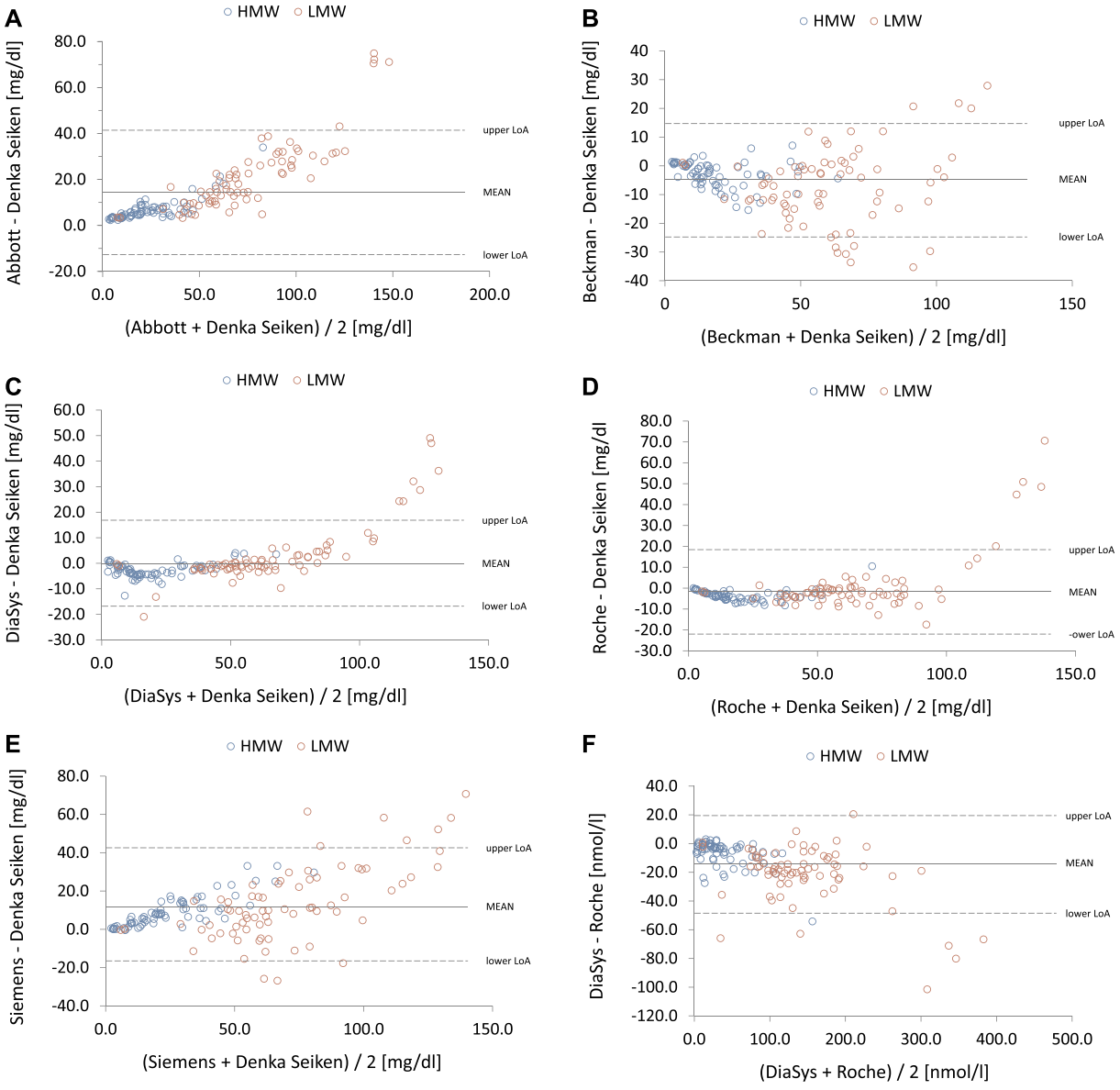

Fig. 1. Bland-Altman difference plots of Lp(a) assays considering the apo(a) isoform size.
The samples were stratified into groups with high molecular weight (blue circles) and low molecular weight (red circles) apo(a) isoforms as described in the Materials and Methods section. To obtain Roche TinaQuant converted, the results from the Roche TinaQant assay (in nmol/l) were converted into mg/dl using a factor of 0.4167 as suggested by the manufacturer. HMW, high molecular weight; LMW, low molecular weight.

4. Discussion

This systematic comparison reveals substantial differences between contemporary commercial assays to measure Lp(a). While the imprecision of all assays was within an acceptable range, the actually obtained concentrations due to calibration differed most likely due to biases. The assays applied in this study produced remarkably different values obtained for the PRM-1 reference standard, which has assigned concentrations of 43.3 mg/dl or 96.6 nmol/l, respectively. In addition, by examining differences plots in detail, we noticed that the differences between the assays were not proportional, but rather varied across the concentration ranges.

We conclude from these analyses that the apo(a) size variation may not conclusively explain the key finding of our study, namely a very consistent positive bias at high Lp(a) concentrations of several assays (Abbott Quantia, DiaSys 21 FS a, Roche TinaQuant converted, Siemens N Latex) in relation to the Denka Seiken. We therefore speculate that the major “overestimation” of Lp(a) at high Lp(a) results from the use of the five independent reference standards across the concentration range of Lp(a). Evidently, these materials may have been assigned different Lp (a) concentrations by the manufacturers. According to Marcovina and Albers [14], the turbidimetric Denka Seiken assay relies on five independent reference standards, each containing an apo(a) isoform distribution representative for the respective concentration range, i.e. the high-level calibrators contain small isoforms and the low-level calibrators containing large ones. Marcovina and Albers claimed from the results of a comparative study using their isoform-independent sandwich enzyme linked immunoassay as the reference that the turbidimetric Denka Seiken assay was largely independent of the apo (a) size polymorphism, despite the fact that the polyclonal antibody used in the latter assay recognizes a repetitive (K4 type (2) epitope [14]. If this was true, then the turbidimetric signal per molecule of Lp(a) would be lower at high concentrations of Lp(a) and the high-level reference standards should already have been “up-calibrated” to compensate for the isoform dependence of the polyclonal antibody. In the case of those assays yielding a positive bias at higher apparent Lp(a) (Abbott Quantia,

Table 6
Relative differences (in %) of Lp(a) concentrations in 144 samples obtained with commercial assays according to molecular weight of apo(a).

A / B		Denka Seiken (mg/dl)	Abbott Quantia (mg/dl)	Beckman (mg/dl)	DiaSys 21FS a (mg/dl)	DiaSys 21FS b (nmol/l)	Roche TinaQuant (nmol/l)	Roche TinaQuant (mg/dl)	Siemens N Latex (mg/dl)
Denka Seiken (mg/dl)	HMW (n=72) LMW (n=72)		34.3 ± 16.2 27.1 ± 10.8	-12.4 ± 26.9 -14.6 ± 21.5	-21.6 ± 29.5 -0.8 ± 19.8			-24.6 ± 11.9 -2.8 ±13.7	32.6 ±17.3 15.2 ±23.0
Abbott Quantia (mg/dl)	HMW (n=72) LMW (n=72)	-34.3 ± 16.2 -27.1 ± 10.8		-46.0 ± 22.5 -41.0 ± 21.3	-53.4 ± 28.7 -27.5 ± 19.0			-57.5 ± 17.3 -29.7 ± 11.6	-1.6 ± 24.4 -11.8 ±18.1
Beckman (mg/dl)	HMW (n=72) LMW (n=72)	12.4 ± 26.9 14.6 ± 21.5	46.0 ± 22.5 41.0 ± 21.3		-8.4 ± 35.9 13.8 ± 27.5			-11.9 ± 28.7 11.9 ± 23.2	43.3 ± 36.6 28.9 ± 33.3
DiaSys 21 FS a[b] (mg/dl)	HMW (n=72) LMW (n=72)	21.6 ± 29.5 -0.8 ± 19.8	53.4 ± 28.7 27.5 ± 19.0	8.4 ± 35.9 -13.8 ± 27.5				-2.5 ± 28.4 -2.0 ± 18.6	53.0 ± 31.6 15.7 ±27.4
DiaSys 21 FS b[b] (nmol/l)	HMW (n=72) LMW (n=72)						-28.0 ± 49.3 -17.4 ± 24.9		
Roche TinaQuant (nmol/l)	HMW (n=72) LMW (n=72)					28.0 ± 49.3 17.4 ± 24.9			
Roche TinaQant converted[c] (mg/dl)	HMW (n=72) LMW (n=72)	24.6 ± 11.9 2.8 ±13.7	57.5 ± 17.3 29.7 ± 11.6	11.9 ± 28.7 -11.9 ± 23.2	2.5 ± 28.4 2.0 ± 18.6				55.9 ± 17.5 17.9 ± 20.4
Siemens N Latex (mg/dl)	HMW (n=72) LMW (n=72)	-32.6 ±17.3 -15.2 ±23.0	1.6 ± 24.4 11.8 ±18.1	-43.3 ± 36.6 -28.9 ± 33.3	-53.0 ± 31.6 -15.7 ±27.4			-55.9 ± 17.5 -17.9 ± 20.4	

[a] The entries contain means with standard deviations (in %), high molecular weight apo(a) (HMW, top row) and low molecular weight apo(a) (LMW, bottom row). The differences were calculated according to the following formula: Difference (%) = [B - A] * 100/[(A + B)/2].
[b] Diasys 21 FS a and Diasys 21 FS b are independent assays, each using five calibrators provided by the manufacturer, the two assays therefore provide differenr results.
[c] Results from the Roche TinaQant, which are in nmol/l, were converted into mg/dl using Lp(a) (mg/dl) = Lp(a) (nmol/l) * 0.4167 as suggested by the manufacturer of the assay.

DiaSys 21 FS a, Roche TinaQuant converted, Siemens N Latex) such a compensation may have even been overdone in the high-level calibrators. Alternatively, “up-calibration” of the Denka Seiken high level standard may have been insufficient. Because the protocols for the assignment of the calibrators in different assays are not public, this explanation of course needs to remain speculative and, importantly, we believe that none of the methods under examinations should be coined as a reference method.

Also, beyond the methods examined here, there is until today no widely accepted commercial gold-standard method for the measurement of Lp(a) nor has a consistent standardization procedure been established. Therefore, Our study was not designed to decide which commercial Lp(a) assayis preferable. Our observations much more demonstrate that further intense international efforts to harmonize the of Lp(a) assays amongst providers of reagents are needed. In the nearer future, the measurement of the apo(a) molar concentrations by liquid chromatography – mass spectrometry using targeting appropriate signature peptides might provide another option that could be employed as a reference method to resolve the methodical issues uncovered by the current study. Efforts along this line are currently underway [22].

It has been suggested that substantial reductions of Lp(a) plasma concentrations starting from very high concentrations at baseline would be needed to demonstrate a cardiovascular benefit [23,24]. For this purpose it appears crucial to measure Lp(a) levels reliably in particular at high levels.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

HS, GK, WM, TG designed the study; HS, TS, BD, GE, WM collected data; HS, HD, GK, WM, TG analysed and interpreted data; HS, WM performed statistical analysis; HS, TG, WM wrote the manuscript; TS, BD, HD, GK, MH validated, reviewed and edited the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.08.015.

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