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Urinary apo(a) discriminates coronary artery disease patients from controls

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Abstract

Increased plasma lipoprotein (a) (Lp(a)) levels are associated with premature cardiovascular diseases and stroke. Since Lp(a) immune reactivity is found in urine we compared urinary apolipoprotein (a) (apo(a)) with plasma Lp(a) levels in 116 patients suffering from angiographically proven coronary artery diseases with that of 109 controls.

Urinary apo(a) investigated by immuno blotting, revealed a distinct apo(a) fragmentation pattern with molecular weights between 50 and 160 kDa. Apolipoprotein B however was not secreted into urine. Lp(a) and apo(a) were measured by a fluorescence immuno assay. Within single individuals, urinary apo(a) levels correlated significantly with creatinine (Rho, 0.98; $P < 0.0005$). Medians and 25/75 percentiles of urinary apo(a) in coronary artery disease (CAD) patients were 5.70, 3.25 and 10.35 μg/dl and in controls 2.64, 1.43 and 3.50 μg/dl respectively. At cut-off levels of 30 mg/dl for plasma Lp(a) and 10 μg/dl of urinary apo(a) respectively, both paramenters showed comparable sensitivities (33.8% vs. 26.7%), yet the specificity (76.1% vs. 91.7%) and the positive predictive value (60.0% vs.76.4%) of urinary apo(a) were much higher. In receiver-operating characteristic plots, urinary apo(a) was much more sensitive at high specificities i.e. greater than 60% as compared to Lp(a). Urinary secretion of apo(a) fragments normalized to creatinine is stable in a given individual and significantly associated with coronary artery disease.

Keywords: Atherosclerosis; Plasma Lp(a); Risk factor; ROC-plot

1. Introduction

Lipoprotein (a) (Lp(a)), is a cholesteryl-ester rich lipoprotein of unknown function consisting of an LDL-like core to which apolipoprotein(a) (apo(a)) is disulfide linked. Apo(a) is a glycoprotein with approximately 29% (w/w) carbohydrates [1]. It exhibits a characteristic structure consisting of numerous kringle-IV (K-IV) repeats, one kringle-V and a protease domain all of which possess high homology to corresponding structures in plasminogen (Plg) [2]. Apo(a) exists in multiple genetically determined isoforms with molecular weights ranging from approximately 350 to 1000 kDa. Presently, more than 30 different apo(a) isoforms are known, which differ from each other by one additional 'repetitive K-IV'. One repetitive K-IV has a theoretical molecular weight including sugars of 16 kDa. The number of K-IV repeats is inversely correlated with the plasma Lp(a) concentration [3,4]. Plasma Lp(a) levels in humans differ from less than 1 mg/dl to over 200 mg/dl, and these levels are reported to be under strict genetic control [3,5].

There is discussion on a possible causal link of Lp(a) with the atherosclerotic process. Supportive evidence stems from observations that Lp(a) accumulates in atherosclerotic plaques [6], stimulates smooth muscle cell proliferation [7], avidly binds to arterial proteoglycans [8] and fibronectins [9] and promotes cholesterol accumulation in cells [10]. Because of the structural

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homology with plasminogen, apo(a) was suggested to interfere at several steps with the fibrinolytic process [11].

Several studies have shown that increased plasma Lp(a) levels are strongly associated with coronary and cerebral artery disease [12–20]. It is, however, of interest to note that in case-control studies Lp(a) turned out to be a much stronger risk indicator than shown in prospective trials [21–23]. Increased plasma Lp(a) was also reported not to be linked to cardiovascular diseases in all ethnic groups. In African-Americans for example, Lp(a) was not an independent risk factor for coronary artery disease (CAD) [24].

Recently, Mooser et al. reported on the secretion of kringle-IV- containing fragments in human urine [25]. We report here that patients suffering from CAD excrete significantly higher amounts of apo(a) fragments into urine as compared to controls. The concentration of urinary apo(a) fragments was significantly correlated to serum Lp(a) in both normal and CAD patients.

2. Patients and controls

Consecutive male (84) and female (32) patients from regions of Vienna and Lower Austria who were admitted for diagnostic coronary angiography to our department were included in this study. Patients referred for diagnostic coronary angiography or angioplasty for typical clinical indications, including evaluation of chest pain, exertional angina pectoris, postinfarction angina or follow-up of postangioplasty symptoms were considered eligible for the study. Only those who suffered from at least one coronary artery stenosis a greater than 70% diameter as proven by quantitative angiography were selected. Subjects with angina at rest, acute myocardial infarction (onset <4 days before angiography), valvular disease, renal failure, diabetes mellitus, liver diseases, congestive heart failure, or alcohol intake greater than 20 g/day were excluded.

Of the 116 patients studied, 38 suffered from single-vessel disease, 30 from double-vessel and 48 from triple or multi-vessel coronary artery disease. Seventy three patients had a previous history myocardial infarction and 59 had hypertension. Thirty one patients were smokers. Twenty four patients were on lipid lowering therapy with lovastatin, 20–40 mg/day or bezafibrate 400 mg/day or gemfibrozil, 500 mg/day. Ninety two patients received aspirin (100 mg/day), 32 calcium antagonists, 54 ACE inhibitors, 55 β-blockers and 97 nitrates.

The control group consisted of 109 clinically healthy individuals (78 males and 31 females) participating in a health survey programme. Controls were matched for age, gender and socioeconomic status. There were 37 smokers in the control group. Controls had no clinical signs of cardiovascular diseases. Apart from occasional pain relievers, the controls did not take any medication. Exclusion criteria for controls were alcohol intake greater than 20 g/day.

2.1. Collection of samples

Blood and urine samples were collected before coronary angiography. Blood was drawn in the morning after a 12 h fasting period, allowed to clot at room temperature for 20 min and submitted immediately to the central laboratory for analysis of lipids, lipoproteins and routine parameters. Urine was also obtained in the morning and tested in the central laboratory for creatinine. Aliquots of urine and serum were stabilized with EDTA (1 mg/ml), frozen immediately at -20°C and stored up to 1 month before Lp(a) and apo(a) were analysed by DELFIA. In preliminary experiments we ascertained that freezing and thawing did not affect the results. Also storage of urine for up to 24h at room temperature had no influence on the banding pattern in SDS-PAGE.

3. Material and methods

3.1. Electrophoresis and Western-blotting

Polyacrylamide gel electrophoresis was carried out in 3.5–13% gradient gels containing 0.1% SDS, prepared in our laboratory using the Mini-protean electrophoresis system from BioRad as described [26,27]. Samples were mixed with SDS (final concentration 10 g/l) in the absence or presence of DTT and heated for 5 min at 100°C. After electrophoresis, proteins were transferred to a nitrocellulose membrane by electroblotting overnight at 4°C in 10 mmol/l Tris-HCl and 40 mmol/l glycine buffer, pH 7.4. To avoid background staining, the membrane was blocked for 60 min in 5% (w/v) powdered skim milk, then incubated for 3 h with affinity-purified rabbit anti-apo(a) antiserum diluted 1:1000 [26,27], washed extensively in Tris-buffered saline (TBS, pH 7.4) containing 0.05% (w/v) Tween-20, and incubated for 2 h with horseradish peroxidase-labelled protein-A. The membrane was further washed as described above followed by incubation with ECL Western blotting detection reagent (Amersham) for 1–2 min and subjected to autoradiography according to the manufacturer's instructions (Amersham). In some cases the monoclonal antibody (MAB) 1A2, kindly provided by H. Dieplinger from the laboratory of G. Utermann, Innsbruck, was used for apo(a) detection. MAB 1A2 is specific for K-IV and recognizes a linear epitope on the repetitive K-IV as well as on 'unique' K-IV types 2, 3, 4, 6 and 7 [28]. MAB 1A2 does not cross react with plasminogen. With this immunoblotting method, less than 5 ng of apo(a) can be detected.

3.2. Quantitation of Lp(a) and apo(a) by DELFIA

Quantitations were carried out by a sandwich assay on the DELFIA system (LKB-Pharmacia), as described in detail previously [26,27,29]. In brief, a polyclonal affinity purified antibody from rabbit, produced in our laboratory that had been immuno affinity purified by passing over a column loaded with plasminogen, was used to coat 96-well Costar plates. The purified antibody was free of any detectable cross reactivity against plasminogen, as tested by Western blot analysis using Glu_1-plasminogen, or other plasma constituents. Non-specific binding sites were blocked with 250 μl of 0.5% (w/v) bovine serum albumin (BSA) for 30 min. Two hundred μl aliquots of the samples were added to the wells and incubated for 2 h at 20°C. After three successive washing steps with 50 mM Tris-HCl, pH 7.7, the polyclonal antibody against apo(a), labelled with Europium (Eu), was added to the wells and incubated additionally for 2 h at 20°C. Excess antibody was removed by two further washing steps with 50 mM Tris-HCl, pH 7.7. 200 μl of enhancement solution (Pharmacia, Uppsala) was added, and fluorescence was determined in a DELFIA reader after 15 min. For the determination of total apo(a), Eu-labelled polyclonal (POAB) anti-apo(a) from rabbit was used (a:a DELFIA) and in some cases, the K-IV-specific monoclonal anti-apo(a), 1A2 was used as a detection antibody. Apo(a):apo B complexes were measured using Eu-labelled antibody against apo B from rabbit as detection antibody as previously described (a:B DELFIA) [29]. For measuring total apo B, a 'B:B' DELFIA was designed accordingly. Standard curves were produced with the Lp(a) 'reference standard' from Immuno Diagnostika (Lot # 2900/170). This standard was calibrated with freshly purified Lp(a) as well as with recombinant apo(a) containing 18 K-IV repeats and expressed in COS-7 cells [26,27], as primary standard. Standard curves of Lp(a) and of recombinant apo(a) were parallel. Using this procedure, 15.01% of Lp(a) mass in the reference standard # 2900/170 consisted of apo(a). The assay was linear between 1 and 100 ng of apo(a) per well; the within-run coefficient of variation was less than 3%. Plasma samples were diluted 1500–3000 fold and urine samples were diluted 10–50 fold.

3.3. Determination laboratory parameters

Creatinine was measured by the Jaffé Method using commercial assay kits from Boehringer. Cholesterol, and triglycerides were measured enzymatically using the assay kits from Boehringer. HDL-cholesterol (-C) was measured from the supernatant after precipitation with polyethylene glycol (Reagent A from Immuno AG, Vienna). LDL-C was calculated according to the Friedewald equation.

3.4. Reagents

All chemicals were reagent grade from E. Merck,if not otherwise stated.

3.5. Statistical analyses

For data analysis, we used the Statistical Package for Social Sciences (SPSS/MAc +). For serum lipids, mean values $\pm$ S.E.M. were calculated and analyzed by a one-way variance test (ANOVA). A students t-test was applied to assess significant differences of continuous variables among groups. Comparison of serum Lp(a) and urine apo(a) values among groups was performed by the Wilcoxon test or by ANOVA after logarithmic transformation of values. Correlations of serum apo(a) and urine apo(a) values were performed by the Spearmann Rank Correlation Test. Receiver-operating characteristic (ROC) plots and analyses were performed according to Beck and Schultz [30], and Zweig and Campbell [31] in order to evaluate the discriminative power of plasma Lp(a) versus urinary apo(a).

4. Results

Table 1 lists some of the demographic data of the studied CAD patients and controls. There was no difference in age, sex, smoking and drinking behaviour. As expected, the CAD patients had higher triglyceride, total cholesterol, LDL-C and lower HDL-C values as compared to the control groups (192 vs. 107 mg/dl; 220 vs. 190 mg/dl; 149 vs. 125 mg/dl and 35 vs. 47 mg/dl respectively). All differences were statistically highly significant ($P < 0.001$) (Table 2).

First we set up experiments to characterize urinary apo(a). Individual human urine samples were concentrated by pressure dialysis approximately 100-fold (molecular weight cut-off filters 5 kDa) and subjected to SDS-PAGE followed by Western blotting. Fig. 1 exhibits the banding patterns obtained from urine of three individuals with apo(a)-phenotypes B/S2; S3/S4 and S1/S1 according to the nomenclature of Utermann

Table 1
Demografic data of the coronary artery disease patients and of controls

	CAD-Patients	Controls	Significance
Number (m/f)	116 (84/32)	109 (80/29)	ns
Age	63 ± 11	58 ± 16	ns
BMI	24.2 ± 3.3	23.1 ± 3.5	ns
Smokers (n)	31	37	ns
Alcohol consumption	<20 g/day	<20 g/day	ns

n, number of smokers; ns, not significant.

Table 2
Lipid and lipoprotein concentrations from 116 CHD patients and 109 matched controls. The values are given in mg/dl and are means ± S.D.

	Triglycerides	Cholesterol	LDL-C	HDL-C
CHD-patients	192.2 ± 89.2	219.8 ± 47.6	148.5 ± 38.2	34.7 ± 11.8
Controls	106.9 ± 53.6	189.8 ± 43.3	124.7 ± 33.4	46.6 ± 14.6
P-value*	0.0001	0.0001	0.0001	0.0001

*, Tested by ANOVA.

et al. [3]. It is noteworthy that the migration of different apo(a) bands of the three samples is very similar or almost identical, yet pronounced differences were observed in the relative intensities of bands. The apparent molecular weight of the major fragments were 50–160 kDa. Minor fragments not seen in the blot were between 30 and 50 kDa and greater than 160 kDa. The banding pattern was indistinguishable whether K-IV specific monoclonal antibody or polyclonal anti-apo(a) antibody was used for detection. We tested this method with urine samples from more than 30 healthy volunteers with various kinds of apo(a) phenotypes and obtained in all cases, comparable results.

In subsequent experiments, normal and 100-fold concentrated urine samples from several donors were subjected to B:B, a:B and a:a DELFIA assays. Neither by

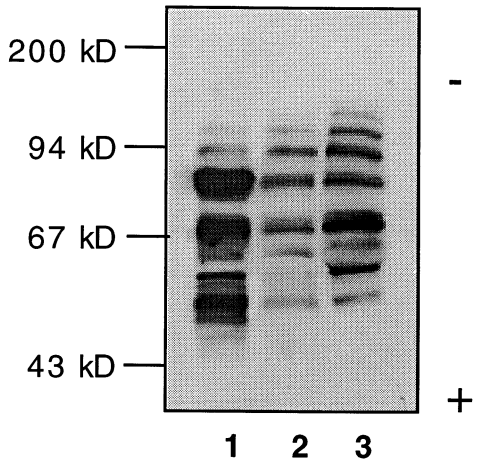

Fig. 1. Immunoblot analysis of apo(a) fragments secreted into urine. Urine from three different healthy donors (1–3) with the apo(a)-phenotypes S1/S1, S2/S4 and B/S3 according to Utermann (3) were concentrated 100-fold and 10 μl of sample each were separated on SDS electrophoresis (3.5–13% polyacrylamide gel) and transblotted to nitro cellulose. The blots were incubated with monoclonal antibody against apo(a) (1A2) which is specific for K-IV and does not cross react with plasminogen. Bands were visualised by the ECL method (see methods section). The migration of marker proteins is indicated at left.

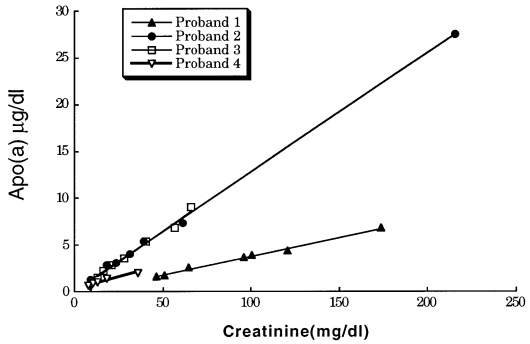

Fig. 2. Correlation of urinary apo(a) levels with creatinine concentration. Urine samples from four healthy volunteers with Lp(a) concentrations of 1: 21.5 mg/dl; 2: 70.6 mg/dl; 3: 55.8 mg/dl and 4: 14.4 mg/dl were obtained on seven different occasions within one month. Lp(a) was measured by DELFIA and correlated with the urinary creatinine concentration.

B:B nor by a:B DELFIA did we observe any measurable amount of apo B containing lipoproteins including intact Lp(a). Considering the sensitivity of these assays we concluded that the content of apo B, if present at all, had to be less than 0.1 μg/dl. Next we adapted the a:a DELFIA for urine samples and found that by diluting urine 10–50 fold instead of 3000 fold as used for serum, we were in the linear range of the standard curve. Urine was assayed at different dilutions and the DELFIA signals were plotted against the apo(a) concentrations; the obtained curve was parallel to the standard curve. Moreover, by spiking urine with Lp(a) or recombinant apo(a) and assaying the samples by DELFIA, we obtained in all cases yields in the range between 94 and 106%. In some cases, apo(a) was measured directly in fresh urine as well as in urine from the same donor concentrated 100-fold by pressure dialysis. The obtained values for diluted and concentrated urine were within the experimental error indicating that our assay was not biased by matrix effects. Thus we were confident that our DELFIA measured urine apo(a) correctly.

In preliminary experiments apo(a) was quantitated in the urine of four volunteers at several time intervals in a 4-week period and large concentration differences were found within single donors. If on the other hand urinary apo(a) levels were normalized to creatinine, the values were surprisingly constant. Fig. 2 exhibits the relationship of the apo(a) concentrations with that of creatinine in urine measured in four different individuals with serum Lp(a) levels in the range of 14–70 mg/dl. While there was a large variation in the absolute apo(a) concentrations over time, urinary apo(a) levels correlated highly significantly with creatinine ($R > 0.98$, $P < 0.0005$). For the following studies urine apo(a) levels were therefore normalized to 1.13 μmol/l (100 mg/dl) of creatinine.

Table 3
Plasma Lp(a) and urinary apo(a) values of CHD patients and of controls

	Plasma Lp(a) (mg/dl)				Urine apo(a) (μg/dl)[a]		
Group	*n*	25%	50%	75%	25%	50%	75%
CHD	116	10.00	18.00	49.50	3.25	5.70	10.35
Controls	109	5.75	11.85	27.50	1.43	2.64	3.50
Z[b]			−12.85			−12.61	
P[b]			0.0001			0.0001	
P[c]			0.003			0.0005	

[a], Normalized to 1.13 mmol/l (100 mg/dl) creatinine.
[b], Tested by Wilcoxon signed rank test: CHD versus controls.
[c], Tested by ANOVA after logarithmic transformation of data: CHD vs. controls.

Urinary apo(a) was then measured in 116 coronary heart disease (CHD) patients and in 109 matched controls (Table 3). Median plasma Lp(a) concentration was significantly higher in patients (18.00 mg/dl) versus controls (11.85 mg/dl). This was also true for the corresponding 25- and 75-percentiles. Urinary median apo(a) values normalized to creatinine in patients and controls were also significantly different: 5.70 and 2.64 μg/dl respectively. It was suggested previously that Lp(a) is significantly associated with CAD at concentrations greater than 30 mg/dl [12]. On the basis of the present data we calculated the predictive power of urinary apo(a) in comparison to plasma Lp(a). At cut-off levels of 30 mg/dl for plasma Lp(a) and 10 μg/dl of urinary apo(a) respectively, both paramenters exhibited comparable sensitivities (33.8 vs. 26.7%), yet the specificity (76.1 vs. 91.7%) and the positive predictive value (60.0 vs. 76.4%) calculated according to R.B. Hynes [13] of urinary apo(a) were much higher. To obtain a better insight into the predictive power of these two parameters, receiver-operating chracteristic plots of the two parameters were carried out by plotting the sensitivity versus 1 minus specificity for all possible cut-off levels. As can be seen from Fig. 3, at high specificities (60 to 100%, i.e., 1-specificity 0.0–0.4), urinary apo(a) has a much higher sensitivity than plasma Lp(a). At low specificities (< 60%) which are of little practical use, the two ROC-curves of apo(a) and Lp(a) overlapped.

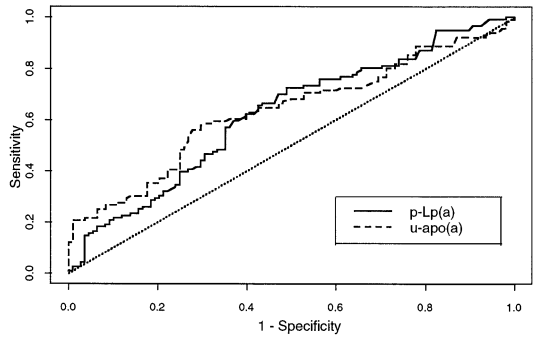

Fig. 3. Receiver-operating characteristic (ROC) plots of plasma Lp(a) and urinary apo(a). ROC-plots, were performed as described in the methods section.

Finally, urinary apo(a) values were correlated with serum Lp(a) (Fig. 4). Using the Spearmann Rank Correlation Test, these parameters correlated significantly in both the CHD patients and control group ($P <$ 0.0001).

5. Discussion

Several recent studies suggest that specific metabolic steps are involved in the biosynthesis and assembly of Lp(a) [26,32–34]; apo(a) is formed probably exclusively in the liver and secreted as a glycoprotein. The residence time within the cell and the efficiency of secretion is strongly influenced by the apo(a) size, explaining partly that individuals with large apo(a) isoforms have significantly lower plasma Lp(a) levels as compared to individuals with small isoforms [35]. Secreted apo(a) interacts with LDL in two steps forming mature Lp(a).

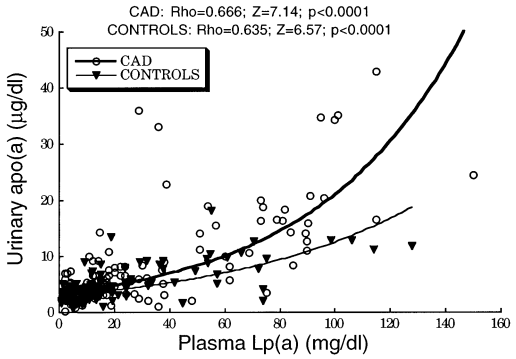

Fig. 4. Correlation of plasma Lp(a) concentrations with urinary apo(a). Lp(a) was measured in fasting plasma from 116 CAD patients as well as from 109 control subjects and correlated with the urinary apo(a) content expressed in μg/dl normalized to 1.13 μmol/l creatinine. The correlations were calculated by the Spearmann rank correlation test. Exponential regression lines are shown for each group separately.

This interaction takes place in part already at the surface of liver cells [36] and later also in circulating blood whereby one or few distinct unique K-IV's form a dissociable complex with lysine or proline or groups of B-100 [27,33,37]. In a second step, a disulfide bridge is formed between cysteine 4057 in apo(a) and most probably Cys 4326 in B-100 [27,33–37]. It also appears that the affinity of apo(a) to B-100 is much higher for LDL than for IDL or VLDL which probably explains the great similarity in core lipid composition between LDL and Lp(a).

In contrast to the biosynthesis and assembly, little has been reported regarding the mode or site of Lp(a) catabolism. Lp(a) binds weakly to the LDL-receptor in vitro yet in vivo the role of the LDL-receptor pathway in Lp(a) catabolism is still a matter of controversity [38–46]. It is also unclear whether Lp(a) is catabolized as a whole lipoprotein or possibly degraded prior to uptake or excretion. Looking at studies in rabbits, it appears that the liver and kidney are primarily involved in Lp(a) removal [44]. It was therefore interesting to learn from the report of Oida et al. [47] that Lp(a)/apo(a) is secreted into urine. Oida et al. [47] studied mainly patients with kidney diseases and reported that these patients secrete significantly less Lp(a) per day than controls. Urinary apo(a) was only poorly defined. In the most recent publication by Mooser et al. [25] urinary apo(a) was characterized further and it was demonstrated that fragments of apo(a) containing only K-IV of type 1 and -2 repeats and perhaps type-3 and -4 are secreted with molecular weights ranging from 85–215 kDa. The C-terminal portion of apo(a) starting beyond K-IV type-4 is missing in urine.

In our study we show that urinary apo(a)-immune reactivity is caused by major apo(a) fragments with apparent molecular weights ranging from approx. 50 to 160 kDa. Minor bands were also present at lower and higer molecular weights. Thirty healthy individuals with large variations in their urinary apo(a) content, and with different apo(a) phenotypes exhibited very similar qualitative Western-blot patterns with major differences in the relative intensities of bands. All fragments shown in Fig. 1 reacted with polyclonal anti-Lp(a) as well as with the K-IV specific monoclonal antibody 1A2, which does not cross-react with K-IV of plasminogen. This observation is consistent with findings published by Mooser et al [25]. We also quantitated in some patients and controls serum Lp(a) and urinary apo(a) values by the a:a DELFIA using monoclonal antibody 1A2 on one hand and polyclonal anti-apo(a) from rabbit on the other hand and found comparable results (r, 0.98; mean values differed by $< 4\%$). For subsequent studies we therefore selected the polyclonal antiserum against Lp(a).

In this study we measured urinary apo(a) secretion in 116 CAD patients compared with 109 control individuals. The constant secretion rates of apo(a) served as a basis for our subsequent calculations. For ethical reasons it was not possible to keep all patients from taking lipid lowering drugs. The proportion of this group within the CHD patients, however, was relatively small. In any case, we performed additional calculations omitting this subgroup and found comparable results (data not shown). The intriguing observation of this work was that CHD patients excreted more than twice as much apo(a) into urine as compared to controls. Although urinary apo(a) was highly significantly correlated to plasma Lp(a) concentrations, urinary apo(a) had a higher predictive power than plasma Lp(a) using cut-off levels of 10 μg/dl and 30 mg/dl respectively.

ROC-plots for plasma Lp(a) and urinary apo(a) gave some further insight into the predictive power of these two parameters (Fig. 3). Taking all cut-off levels together, the two parameters were comparable as the area under the curves were not significantly different (apo(a): $W = 0.6341$; Lp(a): $W = 0.6191$ relative units). At higher specificities which are normally used for discrimiations, i.e., 60–100%, urinary apo(a) exhibited markedly higher sensitivities.

Our results from Fig. 4 also demonstrate a highly significant correlation between plasma Lp(a) and urinary apo(a). It is worth noting that Oida et al. [47] observed neither a correlation of plasma Lp(a) with apo(a) values in urine, nor a correlation of urinary apo(a) with creatinine. The reason for this deviation from our results may reside in differences of the assay and/or reference material used in the two studies, or in the fact that Oida et al. [47] did not normalize their urinary apo(a) values to creatinine. Concerning the correlation with creatinine, it must be emphasized that a correlation with urinary apo(a) was only apparent within single subjects studied at different occasions, but not in the collective as a whole.

Looking at the present results, we can conclude that patients suffering from CAD excrete significantly higher amounts of apo(a) into urine than controls and that urinary apo(a) is a valuable predictor for CAD. Although we are aware of the fact that case control studies like this must be regarded with caution, this study could be a basis for larger prospective trials. Using urinary apo(a) as a marker for CAD has the advantage of easier sampling compared to plasma samples.

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References

[1] Morrisett JD, Gaubath JW, Knapp RD, Guevara JG. Structural properties of apo(a): a major apoprotein of human lipoprotein(a). In: Scanu AM (ed). Lipoprotein(a), San Diego, NY: Academic Press, 1990;53–74.

[2] Mc Lean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM. cDNA sequence of human apolipoprotein(a) in homologous to plasminogen. Nature 1987;330:132–137.

[3] Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotypes: inheritance and relation to Lp(a)-lipoprotein. J Clin Invest 1987;80:458–465.

[4] Lackner C, Boerwinkle E, Leffert CC, Rahnig T, Hobbs HH. Molecular basis of apolipoprotein(a) isoform heterogeneity as resolved by pulsed-field gel electrophoresis. J Clin Invest 1991;87:2153–2161.

[5] Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. J Clin Invest 1992;90:52–60.

[6] Smith EB, Cochran S. Factors influencing the accumulation in fibrous plaques of lipid, derived from low-density lipoproteins: preferential immobilisation of Lp(a). Atherosclerosis 1990;84:173–181.

[7] Grainger DJ, Kirchenloke HL, Metcalfe JC, Weissberg PL, Wade DP, Lawn RM. Proliferation of human smooth muscle cells promoted by Lp(a). Science 1993;260:1655–1658.

[8] Bihari-Vaga M, Gruber R, Rotheneder M, Zechner R, Kostner GM. Interaction of Lp(a) and LDL with glycosamino glycans and proteoglycans from human aorta. Arteriosclerosis 1988;8:851–857

[9] Salonen EM, Jauhiainen M, Zardil F, Vohevi A, Ehnholm C. Lp(a) binds to fibronectin and has serine protease activity, capable of cleaving it. EMBO J 1984;8:4035–4040.

[10] Bottalico LA, Keesla GA, Fless GM, Tobas I. Chosesterol loading of macrophages leads to marked enhancement of native Lp(a) and apo(a) internalization and degradation. J Biol Chem 1993;268:8569–8573.

[11] Miles LA, Plow EF. Lp(a): an interloper into the fibrinolytic system? Thromb and Haemostasis 1990;63(3):331–335.

[12] Kostner GM, Avogaro P, Cazzolato G, Marth E, Bittolo Bon G. Lipoprotein (Lp(a)) and the risk for myocardial infarction. Atherosclerosis 1981;38:51–61.

[13] Haynes RB. How to read clinical journals II: to learn about a diagnostic test. CMA J. 1981;124:703–710.

[14] Armstrong VW, Cremer P, Eberle E, Manke A, Schulze F, Wieland H, Kreuzer H, Seidel D. The association between serum Lp(a) concentrations and angiographically assessed coronary atherosclerosis. Atherosclerosis 1986;62:249–257.

[15] Rhoads GG, Dahlen G, Berg K, Morton NE, Dannenberg, AL. Lp(a) lipoprotein as a risk factor for myocardial infarction. JAMA 1986;256:2540–2544.

[16] Sandholzer C, Saha N, Kark JD, Ress A, Jaross W, Dieplinger H, Hoppichler F, Boerwinkle E, Utermann G. Apo(a) isoforms predict risk for coronary heart disease. Arterioscler Thromb 1992;12:1214–1226.

[17] Marquez A, Mendoza S, Carrasco H, Hamer T, Glueck CJ. High lipoprotein(a) in children from kindreds with parental premature myocardial infarction. Pediatr Res 1993;34:670–674.

[18] Schaefer EJ, Lamon-Fava S, Jenner JL, McNamara JR, Ordovas JM, Davis E, Abolafia JM, Lippel K, Levy RI. Lipoprotein(a) levels and risk of coronary heart disease in men. JAMA 1994;271:999–1003.

[19] Gotoh T, Kuroda T, Yamasawa M, Nishinaga M, Mitsuhashi T, Seino Y, Nagoh N, Kayaba K, Yamada S, Matsuo H, Hosoe M, Itoh Y, Kawai T, Igarashi M, Shimada K. Correlation between lipoprotein(a) and aortic valve sclerosis assessed by echocardiography (the JMS cardiac echo and cohort study). Am J Cardiol 1995;76:928–932.

[20] Zenker G, Költringer P, Boné G, Niederkorn K, Pfeiffer K, Jürgens G. Lipoprotein(a) as a strong indicator for cerebrovascular disease. Stroke 1986;17:942–945.

[21] Ridker PM, Hennekens CH, Stampfer MJ. A prospective study of lipoprotein(a) and the risk of myocardial infarction. JAMA 1993;270:2195–2199.

[22] Cremer P, Nagel D, Labrot B, Mann H, Muche R, Elster H, Seidel D. Lipoprotein Lp(a) as predictor of myocardial infarction in comparison to fibrinogen, LDL cholesterol and other risk factors: results from the prospective göttingen risk incidence and prevalence study (GRIPS). Eur J Clin Invest 1994;24:444–453.

[23] Alfthan G, Pekkanen J, Jauhiainen M, Pitkäniemi J, Karvonen M, Tuomilehto J, Salonen JT, Ehnholm C. Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population based study. Atherosclerosis 1994;106:9–19.

[24] Moliterno DJ, Jokinen EV, Miserez AR, Lange RA, Willard JE, Boerwinkle E, Hillis D, Hobbs HH. No association between plasma lipoprotein(a) concentrations and the presence or absence of coronary atherosclerosis in African-Americans. Arterioscler Thromb Vasc Biol 1995;15:850–855.

[25] Mooser V, Seabra MC, Abedin M, Landschulz KT, Marcovina S, Hobbs HH. Apolipoprotein(a) kringle 4-containing fragments in human urine. J Clin Invest 1996;97:858–864.

[26] Frank S, Krasznai K, Durovic S, Lobentanz E-M, Dieplinger H, Wagner E, Zatloukal K, Cotten M, Utermann G, Kostner GM, Zechner R. High level expression of various apolipoprotein(a) isoforms by 'transferrinfection': the role of kringle-IV sequences in the extracellular association with low density lipoprotein. Biochemistry 1994;33:12329–12339.

[27] Frank S, Durovic S, Kostner GM. Structural requirements of Apo(a) for the Lp(a) assembly. Biochem J 1994;304:27–30.

[28] Dieplinger HD, Gruber G, Krasznai K, Reschauer S, Seidel C, Burns G, Müller HJ, Csaszar A, Vogel W, Robenek H, Utermann G. Kringle 4 of human apolipoprotein (a) shares a linear antigenic site with human catalase. J Lipid Res 1995;36:813–822.

[29] Steyrer E, Durovic S, Frank S, Gießauf W, Burger A, Dieplinger H, Zechner R, Kostner GM. The role of lecithin: cholesterol acyltransferase for lipoprotein(a) assembly. Structural integrity of low density lipoproteins is a prerequisite for Lp(a) formation in human plasma. J Clin Invest 1994;94:2330–2340.

[30] Beck JR, Schultz EK. The use of relative operating characteristic (ROC) curves in test performance evaluation. Arch Pathol Lab Med 1986;110:13–20.

[31] Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–577.

[32] White AL, Rainwater DL, Lanford RF. Intracellular maturation of apoprotein(a) and assembly of lipoprotein(a) in primary baboon hepatocytes. J Lipid Res 1993;34:509–517.

[33] Ernst A, Helmhold M, Brunner C, Pethö-Schramm A, Armstrong WV, Müller HJ. Indentification of two functionally distinct lysine-binding sites in kringle 37 and kringle 32–37 of human apolipoprotein(a). J Biol Chem 1995;270:6227–6234.

[34] Linton MF, Farese RV Jr, Chiesa G, Gross DS, Chin P, Hammer RE, Hobbs HH, Young SG. Transgenic mice expressing high plasma concentration of human apolipoprotein B-100 and lipoprotein(a). J Clin Invest 1993;92:3029–3037.

[35] White AL, Hixon JE, Rainwater DL, Lanford RE. Molecular basis for 'null' lipoprotein(a) phenotypes and the influence of apolipoprotein(a) size on plasma lipoprotein(a) level in the baboon. J Biol Chem 1994;269:9060–9066.

[36] White AL, Lanford RE. Cell surface assembly of lipoprotein(a) in primary cultures of baboon hepatocytes. J Biol Chem 1994;269:28 716–28 723.
[37] Trieu VN, McConathy WJ. A two-step model for lipoprotein(a) formation. J Biol Chem 1995;270:15 471–15 474.
[38] Armstrong VW, Harrach B, Robenek H, Helmhold M, Walli AK, Seidel D. Heterogenity of human lipoprotein Lp(a): cytochemical and biochemical studies on the interaction of two Lp(a) species with the LDL receptor. J Lipid Res 1990;31:429–441.
[39] Steyrer E, Kostner GM. The interaction of lipoprotein Lp(a) with the B/E receptor. J Lipid Res 1990;31:1247–1253.
[40] Krempler F, Kostner GM, Roscher A, Sandhofer F. Studies on the role of specific cell surface receptors on the removal of Lp(a) in man. J Clin Invest 1983;71:1431–1441.
[41] Kostner GM, Gavish D, Leopold B, Bolzano K, Weintraub MS, Breslow JL. HMG CoA reductase inhibitors lower LDL cholesterol without reducing Lp(a) levels. Circulation 1989;80:1313–1319.
[42] Kostner GM, Grillhofer H. Lipoprotein(a) mediates high affinity low density lipoprotein association to receptor negative fibroblasts. J Biol Chem 1991;266:21 287–21 292.
[43] Kostner GM. Interaction of Lp(a) and of apo(a) with liver cells. Arterioscler Thromb 1993;13:1101–1109.
[44] Liu R, Saku K, Kostner GM, Hirata K, Zhang B, Shiom M, Arakawa K. In vivo kinetics of lipoprotein(a) in homozygous Watanabe heritable hyperlipidaemic rabbits. Eur J Clin Invest 1993;23:561–565.
[45] Hofman SL, Eaton DL, Brown MS, McConnathy WJ, Goldstein JL, Hammer RE. Overexpression of human LDL-receptors leads to accelerated catabolism of Lp(a) lipoprotein in transgenic mice. J Clin Invest 1990;85:1542–1547.
[46] Kostner GM. The physiological role of Lp(a) In: Scanu AM (ed). Lipoprotein(a). Inc. Harcourt Brace Javanovich, Academic Press, Publishers 1990;183–204.
[47] Oida K, Takai H, Maeda H, Takahashi S, Shimada A, Suzuki J, Tamai T, Nakai T, Miyabo S. Apolipoprotein(a) is present in urine and its excretion is decreased in patients with renal failure. Clin Chem 1992;38/11:2244–2248.