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Lipoprotein(a): when to measure, how to treat?

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Abstract: Lipoprotein(a) [Lp(a)] is one of the most atherogenic lipoproteins consisting of a core low-density lipoprotein particle and the specific glycoprotein apo(a). Apo(a) is homologous to plasminogen yet in contrast exhibits a specific size polymorphism. This polymorphism is due to the fact that the number of kringle-IV (K-IV) repeats ranges between two and approximately 50. Apo(a) is synthesized almost exclusively in the liver, and there is still some discussion regarding whether the assembly of Lp(a) occurs intracellularly or in the circulating blood. The plasma Lp(a) concentration is markedly skewed to the right and extends from <1 mg/dL to more than 200 mg/dL. Up to 90% of the variance of Lp(a) concentrations may be genetically determined and the Lp(a) concentration correlates inversely with the number of K-IV repeats. In the apo(a) promoter there are numerous response elements for transcription factors and nuclear receptors, whereby the HNF4α binding sequence is the most important one. Activation of FXR causes the dissociation of HNF4α from its response element and in turn a significant down regulation of apo(a) transcription. Recent large epidemiological studies document beyond any doubt that Lp(a) is an independent causal risk factor for coronary heart disease and myocardial infarction. Hence, novel approaches to correct elevated Lp(a) are under investigation. Among the established lipid-lowering drugs, only nicotinic acid lowers Lp(a) in a consistent and clinically relevant fashion, and we recently elucidated the molecular mechanism underlying this effect. Novel medicines in clinical trials include CETP inhibitors, PCSK9 antibodies, the MTP inhibitor lomitapide and antisense oligonucleotides. APO(a)$_{Rx}$®, an antisense oligonucleotide, which is specifically directed against the mRNA for apo(a), has the strongest effect on Lp(a). It offers the opportunity to examine the impact of selective Lp(a) lowering on clinical events. Lp(a) emerged as an important screening parameter to assess the risk for atherosclerosis. Its quantitation in the clinical laboratory had not been standardized for a long period of time. New commercial methods, in particular enzyme immunoassays with monoclonal antibodies that recognize single epitopes in apo(a), or nephelometric and turbidimetric assays hold the potential to warrant comparable results in different laboratories.

Keywords: atherosclerosis; coronary heart disease; drug treatment; fibrinolysis; metabolism; reference values.

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Introduction

Lipoprotein(a) [Lp(a)] has been known since the early 1960s, and its role in atherogenesis has long been controversial. This is largely so because, to date, it has been impossible to allocate any function to it, and especially because hardly any specific therapies exist. Lp(a) consists of a "core" LDL and the specific antigen, apolipoprotein(a) [apo(a)]. The latter is highly homologous to plasminogen, which is why it was thought that apo(a) served functions relative to fibrinolysis. There is now a large number of publications dealing with this issue (see review in [1]) and pointing out pathophysiological effects in coagulation, but in practice these hypotheses gained little importance.

Of much greater interest is the now unequivocally proven causal relationship between elevated Lp(a) and the incidence rates for atherosclerosis, coronary heart disease and stroke [2–4]. It is noteworthy, however, that there are scientific studies that suggest that Lp(a) plasma concentrations increase with age, and/or that 90-year-olds exhibit significantly higher Lp(a) levels than the young population [5].

Lp(a) metabolism

The protein fraction of Lp(a) is composed of two main components: ApoB-100 and apo(a) [6]. ApoB-100 is produced in the liver and is the main component of LDL, the end product of VLDL metabolism. LDL are probably also directly secreted by the liver; however, such LDL have a different composition than those originating from VLDL. Apo(a) consists of 11 unique "kringles IV"'s, which are highly homologous to the kringles IV of plasminogen, and of a varying number of repetitive K-IVs: this is being discussed as the main cause of many discrepancies in the immunochemical determination of Lp(a). Apo(a) also has a kringle V and an inactive protease domain (for further details on the Lp(a) structure, see Ref. [6]). How and where Lp(a) is assembled from these two components is irrelevant to the analysis, but has important implications for the development of Lp(a)-lowering medications and the interpretation of their modes of action. If one mixes recombinant apo(a) with LDL in a test tube, the result, after a short incubation period, will be a lipoprotein that is indistinguishable from native Lp(a), which suggests an extracellular formation. Metabolic studies performed by the group around Hans Dieplinger in Innsbruck showed, by contrast, that the rates of synthesis of the protein components in Lp(a) [ApoB-100 and apo(a)] are identical, while those of ApoB-100 in LDL are different, suggesting an intrahepatic assembly of Lp(a) [7].

It is known that there are not only serious ethnic differences in the plasma concentrations of Lp(a) (the Black population has the highest and Asians have the lowest Lp(a) concentrations [8]), but also within ethnic groups, with varying plasma concentrations of between <1 mg/dL and >200 mg/dL. This is partly due to polymorphisms in the apo(a) promoter and the coding regions, as well as the size polymorphism of the different number of "K-IV *repeats*", with a significant negative correlation between the number of K-IVs and the plasma concentration.

The biosynthesis of apo(a)

The gene locus of apo(a) is located on chromosome 6 (6q26-q276). Apo(a) is synthesized in the same manner as is usual with glycoproteins. The relationship between the number of K-IV repeats and the plasma concentration is explained by the fact that high molecular weight apo(a)'s remain in the cytoplasm much longer than low molecular weight ones, and thus also have a much higher rate of degradation.

However, the synthesis rate of the apo(a) is also determined significantly by the activity of the promoter, and its activation by transcription factors and nuclear receptors. We have been able to show that the apo(a) promoter has binding sites for over 70 transcription factors; among them, HNFs, FXR, PPARs, RXR, SREBPs, CCAAT enhancer, IL-6, as well as a number of others that play an important role in the lipoprotein metabolism [9].

Due to the large number of binding sites for transcription modulators, it can be assumed that the regulation of apo(a) transcription is complex. Meanwhile, we have succeeded in identifying the most important element in the apo(a) promoter that is responsible for the transcription and plasma Lp(a) concentration (Figure 1).

Lp(a) catabolism

Not only is the biosynthesis of Lp(a) still largely in the dark, but so also is the site of degradation or uptake into the cell. In vivo turnover studies involving test animals that themselves do not produce Lp(a) show that more than 50% ends up in the liver and that apo(a) degradation products are excreted via the bile [10]. However, significant radioactivities of labeled Lp(a) also reach the kidney, spleen, lungs and the pancreas. It is unknown whether these tissues are relevant to the degradation in humans. Since the liver represents the main organ of the catabolism of lipoproteins facilitated by the LDL receptor, it was necessary to investigate whether Lp(a) would take a similar path of degradation. In vivo experiments at our laboratory and various working groups, however, indicate that the LDL-R binds only little Lp(a). This is mainly supported by the fact that patients with homozygous FH exhibit a fractional degradation rate similar to healthy controls [11]. Since an understanding of the catabolism of Lp(a) is crucial to strategies for developing Lp(a)-lowering drugs, there have been increased efforts to examine the role of other lipoprotein receptors in the binding of Lp(a). In fact, there is hardly a receptor, including LRP, VLDL-R, asialo-R and various scavenger receptors, which

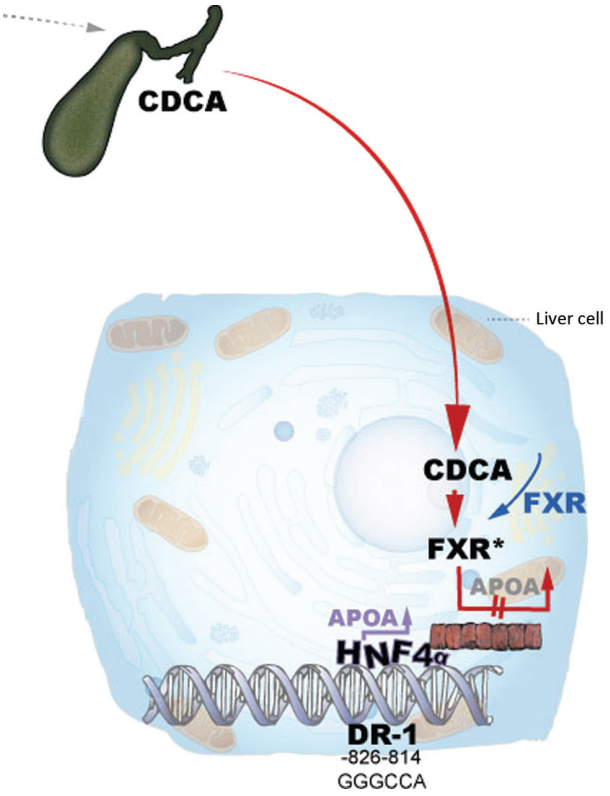

Figure 1: Inhibition of apo(a) transcription by bile acids. Chenodeoxycholic acid (CDOA), the agonist with the highest affinity for FXR in humans, binds and activates the farnesoid X receptor (FXR) and suppresses the binding of HNF4α to the positive transcription element in the promoter reagion – 826 to 814 relative to the transcription start site.

is not considered a "hot candidate" for Lp(a) binding. Unfortunately, all of these results are based on in vitro experiments, which in some cases have little relevance to the in vivo situation. A probably important mechanism of the binding and cellular uptake of Lp(a) is based on the affinity of apo(a) kringles to lysine side groups of cell surface structures. We were thus able to demonstrate that the feeding of Lys analogs, such as tranexamic acid, to transgenic apo(a) or Lp(a) mice increased the concentration of Lp(a) by a factor of 1.5 to 2, which correlated with a decreased cellular uptake [12].

Lp(a) – a risk factor for atherosclerosis, coronary heart disease and stroke

If one enters in MEDLINE the search terms Lp(a) and atherosclerosis or CHD, one will receive more than 1500 hits. It is therefore impossible to consider all of these publications in this context, which is why we focus on the essential. Semi-quantitative analyses of Lp(a) in Scandinavia performed by the discoverer of Lp(a), Kare Berg, showed that individuals with an increased presence of "sinking pre-beta" lipoprotein [corresponding to Lp(a)] exhibited an increased incidence of angina pectoris and coronary heart disease [13]. The first quantitative analyses of Lp(a) were conducted in our laboratory in collaboration with the "Avogaro group" (Venice). In a case-control study of only 183 subjects, we observed that the relative risk (RR) of myocardial infarction – depending on the recognized threshold – increased about twofold [14]. As the upper limit of Lp(a), 30 mg/dL was adopted in connection with this study and subsequently by most working groups. In our first study we were also able to show that an approximately sixfold increase in the risk of myocardial infarction existed when elevated Lp(a) was accompanied by a type IIa phenotype (characteristic of familial hypercholesterolemia). Our observations were followed by countless – some prospective – studies most of which found a significant CHD risk for persons with elevated Lp(a). However, some studies were also negative (see overview in [15]). However, the *mortal dagger* for Lp(a) research came in the form of a publication by Ridker et al. [16]: based on the results of a "nested case–control" study involving test subjects from the prospective "Physician's Health Study" with almost 15,000 participants, no significant association between elevated Lp(a) and the risk of CHD was found (verbatim: *In this prospective study of predominantly middle-aged white men, we found no evidence of association between Lp(a) level and risk of future MI. These data do not support the use of Lp(a) level as a screening tool to define cardiovascular risk among this population.*).

However, Lp(a) research was revived by publications of various working groups in 2009–2011, which included more than 100,000 test subjects/patients and demonstrated significant and obvious causal correlations between elevated Lp(a) and CHD [2–4, 17, 18].

Notable studies over the past 3 years have underscored the importance of Lp(a) as a risk factor for vascular disease: The PROCARDIS CONSORTIUM [19] addressed the long-debated question whether apo(a) isoforms with different numbers of K-IV *repeats* are variously atherogenic; there had been indications in the literature that not only the concentration of Lp(a), but also the size polymorphism had to be taken into account when estimating the risk of atherosclerosis. In the PROCARDIS study Lp(a) was measured in the plasma in nearly 1000 CHD patients and a similar number of control subjects by means of a "latex-enhanced immunoturbidimetric

immunoassay" (see below). Apo(a) isoforms were analyzed by conventional methods by means of SDS-PAGE, followed by immuno-blotting using an isoform standard of the company Immuno AG in Vienna, Austria. Immuno AG has been defunct for years, and the isoform standard is no longer available. The authors calculated the odds ratio (OR) between patients and controls between the first and the fifth quintile of the Lp(a) concentrations before and after adjustment for the number of K-IV *repeats*. In both cases, an OR of 2.05 ($p<0.001$) was found, so it does not matter whether the number of K-IV kringles was taken into account or not. This provides a clear answer to a long discussion, and proves that the size polymorphism affects the atherogenic risk only via its effect on the Lp(a) concentration. Florian Kronenberg penned an editorial on this article, in which he points out that the analysis of SNPs – especially rs41272114, rs10455872 and rs3798220, which have the strongest associations with the Lp(a) concentration – can be neither a surrogate nor a substitute for the number of K-IV *repeats*. More than half of the carriers of small apo(a) isoforms (<22 K-IV *repeats)* and with an increased risk of CHD are not covered by this SNP analysis!

The PROCARDIS Consortium recently published another interesting study in ATVB [20] and presented the question regarding to what extent the LPA null allele (rs41272114) affects the plasma Lp(a) concentration (in heterozygous gene carriers) and determines the risk of atherosclerosis. This study, which included about 8000 CAD patients and controls, found an rs41272114-allele frequency of about 3%. Null allele carriers exhibited highly significantly lower Lp(a) concentrations than control subjects and fewer CHD cases (OR 0.79; $p=0.023$). As is known from studies of the group headed by Gerd Utermann [21], the rs41272114 SNP is a "donor-splice site" mutation and leads to the biosynthesis of a truncated apo(a) with only 7 K-IVs (K-IV 1-7). The absence of K-IV type 9, which also contains the free -SH group for attachment to ApoB-100, requires that the apo(a) fragment is indeed secreted from the liver into the blood, but is very quickly degraded there. The PROCARDIS study, then, clearly showed that people with only one apo(a) isoform have a very large variation in their plasma concentration and a sigmoidal correlation between the number of K-IV *repeats* and plasma Lp(a) levels. However, the cause of this has not been identified yet. Based on their findings, the authors demanded that future epidemiological SNP studies include the rs41272114 polymorphism in order to identify CHD risk factors.

Further evidence for the thesis established in 1981 that Lp(a) was a significant risk factor [14] was provided by the prospective Bruneck study [22] with 826 male and female subjects. A follow-up 15 years later showed that the inclusion of Lp(a), in addition to the Framingham algorithm, improved the C-index by 0.016. The measurement of Lp(a) improved the success rate in predicting CHD by 40%.

Lp(a) and stroke

The question regarding whether Lp(a) may also be used as a predictor for stroke was addressed in numerous publications (see overview in [23]). Recently, a meta-analysis was published that correlated the risk of ischemic stroke in children with plasma concentrations of Lp(a) [24]. Taking into account ten published studies that mostly set 30 mg/dL as a cut-off for elevated Lp(a), the authors found a positive association with a Mantel-Haenszel OR of 4.24 ($p<0.00001$).

As mentioned, the physiological role of Lp(a) is controversial, if not unknown. Sam Tsimikas in San Diego believes the high affinity of Lp(a) for oxidized phospholipids [25] is responsible for its atherogenic effects. It is said that oxidized phospholipids stimulate the formation of pro-inflammatory cytokines and recruit monocytes, which specifically bind modified LDL and transform them into foam cells. This seems to establish a connection to atherogenesis.

However, negatively charged phospholipids are not only a part of oxidized lipoproteins, but also bind with high affinity to beta-2-glycoprotein I (β2-GPI). What is also known about β2-GPI is that it forms a complex with Lp(a). In a recent paper [26], it has been reported, interestingly enough, that serum levels of Lp(a), Ox-Lp(a) and β2-GPI-Lp(a) complexes were higher in stroke patients than in controls (124 patients vs. 64 controls) and that the concentration correlated with the severity of brain stroke. A positive correlation between β2-GPI-Lp(a) and Ox-Lp(a) was also established. These findings must be interpreted to mean that Lp(a) does not neutralize the inflammatory PL by binding them; on the contrary, it should mean that the atherogenic effect of Ox-PL is, in fact, potentiated by Lp(a).

Drug-based control of high Lp(a) levels (see also summary in ref. [27])

Although the studies cited above yield a very strong indication – if not proof – that there is a causal relationship between elevated Lp(a) and coronary heart disease as well as stroke, what is still missing is "final proof", which only intervention studies with specific Lp(a)-lowering medications can deliver. Unfortunately, there are hardly

any medications that target and reduce specifically only Lp(a). The results of epidemiological studies, however, have given rise to intensive work to develop such medications, while all lipid reducers and HDL-boosting drugs in development are examined for their Lp(a)-lowering effect.

There are now a number of suggested treatments for patients with elevated Lp(a) (overview in [15]). Many are recommended based on anecdotal observations, while others are either not very practical or not sufficiently effective. As noted above, not much Lp(a) is metabolized via LDL-R, which is why medications that specifically increase LDL-R activity will achieve hardly any reduction in Lp(a) concentrations. There is even evidence that patients with severely elevated Lp(a) respond to statin therapy with yet another increase in Lp(a) levels [28]. The pathological mechanism behind this observation is not known, unfortunately. The only established method so far for a drastic Lp(a) reduction is apheresis, which also demonstrably reduces the risk of CHD [29]. It is especially recommended for secondary prevention in patients with very high Lp(a) levels.

Another option is the treatment with nicotinic acid preparations. Nicotinic acid (niacin) and/or the acid amide or different extended release and combination products were prescribed in many countries for a long time – mainly because of the HDL-boosting effect. In addition, these preparations have been ascribed Lp(a)-reducing effects, depending on the dose, of up to 30% [30]. We were recently able to explain the molecular mechanism behind the Lp(a) reduction by nicotinic acid: In the *APOA* promoter, there are several cAMP response elements (binding sites) that influence apo(a) transcription [31]. Nicotinic acid interferes in the liver with the binding of cAMP to these elements and reduces apo(a) biosynthesis (see diagram in Figure 2).

As a result of the known side effects of nicotinic acid – especially "flushing" – these preparations are no longer commercially available or rarely prescribed in most countries. This type of medication suffered another setback after the publication of the HPS2-THRIVE study (http://www.thrivestudy.org/): the additional administration of ER niacin/laropiprant did not produce a significant reduction in myocardial infarction or in the incidence of stroke in 25,000 patients.

Selective Lp(a) drugs

Based on our observations that patients with cholestasis have very low Lp(a) levels, which significantly increased again after successful treatment of the underlying disease,

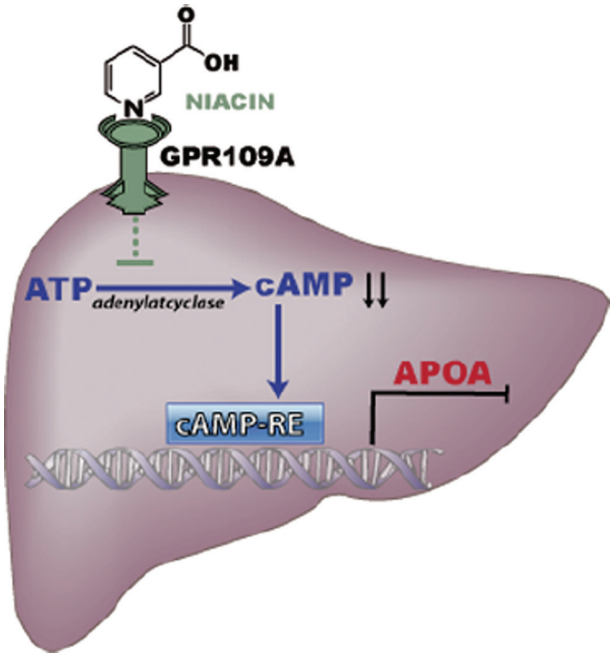

Figure 2: Suggested mechanism of action of nicotinic acid on apo(a) biosynthesis.
The apo(a) biosynthesis is activated by cAMP, which binds to specific "response elements" in the apo(a) promoter. Nicotinic acid inhibits adenylate cyclase, and thus the cAMP concentration in the liver, reducing apo(a) transcription.

we succeeded in identifying FXR as a major nuclear receptor for apo(a) expression (see Figure 1). However, FXR is a pluripotent nuclear receptor that plays an important role not only in bile acid and glucose metabolism, but is also related to LXR, the master regulator of cellular cholesterol metabolism. Negative "feed-back loops" between LXR, FXR and other transcription factors and interleukins are also involved in the control of apo(a) production. Therefore, the use of FXR agonists in long-term drug treatment should be considered with the utmost of caution. Nevertheless, such agonists (and also antagonists) are in development and are being tested, among other things, with respect to Lp(a) reduction. The company Phenex, which specializes in the development of nuclear receptor mimetics, has run clinical trials on the FXR agonist Px-102® since 2011 (http://www.phenex-pharma.com/pdf/PR-Phenex-Phase%20I%20finished_5%20M%20Euros_engl.pdf.). In animal experiments, it has exhibited significant cholesterol-reducing effects, and is also tested for use against liver tumors. We eagerly await further results regarding Px-102® – especially with respect to the effect on Lp(a).

More advanced still are drugs that were developed primarily against high cholesterol and/or to boost HDL levels, such as PCSK-9 inhibitors, CETP activators, MTP inhibitors and thyromimetics.

PCSK-9 inhibitors

In a recent article published in *Circulation*, it was reported that AMG145, the monoclonal antibody against PCSK9 from Amgen® (Thousand Oaks, CA, USA), reduced the Lp(a) concentration by an average of 32%, using a dose of 105 mg Q2W [32]. At such a dose, the authors observed an LDL-C or apoB reduction of 60% or 50%, respectively. Of the 626 (m+f) patients, however, about half had Lp(a) levels below the median of 43 nmol/L. Even though there was a significant positive correlation between the Lp(a)-lowering effect of AMG-145 (140 mg Q2W) and the reduction of LDL-C, it became clear that patients with low Lp(a) exhibited a much more drastic Lp(a) reduction than patients in the 3rd and 4th quartiles of Lp(a). At a dose of 420 mg Q4W, no Lp(a) reduction at all was observed in patients of the 4th quartile. AMG 145 (Evolocumab, Repatha®) is now on the market in Europe and Alirocumab (Praluent®) is expected to follow soon. However, the cost for this treatment will be considerably higher than that of statin therapy.

In a similar study involving SAR236553, the PCSK-9 antibody from the company Sanofi (Gentilly, France), Lp(a) reductions of up to 28.6% on average were observed [33].

These studies can at best be seen as pilot testing for the effect of PCSK-9 inhibitors on Lp(a). They leave a lot of unanswered questions, such as regarding the mechanism of action. It is indeed known that PCSK-9 antibodies increase the LDL-R activity mainly in the liver, and that this receptor has only a relatively low affinity for Lp(a).

CETP inhibitors

CETP is an "exchange-transfer" protein, which catalyzes the exchange of neutral lipids (TG and CE) between VLDL or LDL and HDL. We were able to show years ago that this is also true of Lp(a) and that neutral lipids are also exchanged between Lp(a) and HDL [34], which may be the basis of a possible mechanism of action. Theoretically, this class of drugs would be ideal, as, in particular, those at high risk of a stroke exhibit significantly reduced HDL and elevated Lp(a) [35]. The CETP inhibitors torcetrapib and dalcetrapib were withdrawn due to adverse effects or lack of positive effects; anacetrapib and evacetrapib are in phase III development. Anacetrapib has been repeatedly reported as being capable of lowering Lp(a) by up to 25%. However, important details regarding the effects on Lp(a) are not known.

While other drugs – like eprotirome®, a thyromimetic, lomitapide, an MTP inhibitor from Aegerion (Cambridge, MA, USA) [36], and mipomersen from the class of antisense oligonucleotides against ApoB – do reduce Lp(a) [37], it is far from clear that they will ever be approved for this indication.

The latter class of medications also includes APO(a)$_{Rx}$, which is being developed by ISIS (Carlsbad, CA, USA). We already demonstrated in 2001, in transgenic mice, that using mRNA interference allowed for an almost 100% inhibition of apo(a) synthesis [38]. In fact, ISIS achieved a reduction of plasma Lp(a) levels of up to 95% in a phase I study on patients with 10 mg/dL to almost 100 mg/dL Lp(a) (http://www.isispharm.com/Pipeline/TherapeuticAreas/Cardiovascular.htm#ISIS-APOARx). If ISIS APO(a)$_{Rx}$ were to be approved, this strategy would appear to be the most promising possibility at this moment of a specific, effective and practicable Lp(a) therapy.

When should Lp(a) analyses be performed? (see also [15])

To date, the laboratory-based analysis of Lp(a) has had a rather shadowy existence, especially when considering the argument that there is no viable medication for hyper-Lp(a) patients. It appears that this argument will soon be obsolete, and even if it takes years before the drugs mentioned above are approved, the knowledge of an "additional risk factor" in the risk assessment will be of great importance. Although it was originally reported that the Lp(a) concentration remained constant over time, and could not be lowered by diet or lifestyle changes, it became apparent after the systematic evaluations of individual patients that plasma Lp(a) levels did fluctuate within relatively wide margins. Repeated testing, therefore, is appropriate in the event of elevated levels or borderline values.

In any case, it is recommended that Lp(a) be tested for CHD and stroke patients where the conventional risk factors do not explain the clinical picture. Since high Lp(a) levels can be inherited, testing family members of index patients with elevated Lp(a) is also indicated. We further recommend Lp(a) testing in cases of premature myocardial infarction or stroke and for patients with borderline risk, because they will fall into a higher risk group should Lp(a) become elevated. Since a specific medication for Lp(a) is not currently possible, elevated Lp(a) should trigger a more intensive treatment of the risk factors that can be influenced. Furthermore, the monitoring of Lp(a) is indicated in patients who, despite "state of the art" therapy, exhibit a progressive course of vascular disease, as well as in all FH patients or in connection with genetic dyslipoproteinemia, in groups of patients with reduced HDL, pathologically elevated homocysteine and hemostasis

disorders. Finally, Lp(a) testing is recommended in connection with diabetes mellitus and autoimmune diseases.

According to a consensus of the European Atherosclerosis Society, Lp(a) testing is recommended for patients with a 10-year risk of atherosclerosis above 3%. Particular attention should also be paid to hemodialysis patients and patients with any type of kidney disease. In the latter cases, it is of course important to control the underlying primary disease as much as possible and rigorously treat the modifiable risk factors, including LDL-C, high blood pressure, smoking, high blood sugar and obesity. LDL/Lp(a) apheresis and nicotinic acid preparations have to be taken into consideration for such patients, if practicable, although there is very little evidence from controlled intervention studies.

What to consider when testing Lp(a)

The original laboratory methods, on which the atherogenicity of Lp(a) was based, were radial immunodiffusion, rocket electrophoresis and, coming later, nephelometry. Today, of course, only high through-put methods are in demand – mostly ELISA and DELFIA, nephelometry and turbidimetry. In all these methods, one must be aware that the molecular weight of Lp(a) and apo(a) varies within very wide margins, that Lp(a), as a main component in addition to apo(a), also contains ApoB-100, that Lp(a) has a high affinity to other proteins such as β2-GPI, and especially that there are repetitive structures in apo(a): The number of K-IV repeats varies from two to around 40. With many immunochemical methods, this results in an overestimation of the concentration of Lp(a) with large isoforms and an underestimation of Lp(a) with small isoforms. Finally, the plasma also contains free apo(a) fragments the concentration of which strongly correlates with the plasma Lp(a) concentration. To avoid the problems of Lp(a) analysis, ELISA and DELFIA methods have been developed in which the "capture" antibody can bind, relatively non-specifically, all apo(a) isoforms, while the "detection" antibody either represents a monoclonal antibody (MOAB) that recognizes only one epitope in all apo(a) isoforms, or detects the ApoB component in Lp(a). The latter method comes at the risk that, in hyperlipidemic plasma samples, Lp(a) may bind further LDL particles, which leads to an overestimation of the concentration.

Due to the above-mentioned problems with the standardization of Lp(a) analysis, a panel of experts took it upon itself to address these problems, releasing various reference standards and methodological recommendations. Our research group, too, participated in these experiments involving apo(a)-apo(a) and apo(a)-ApoB DELFIA assays – our results were not always in line with all the members of the rest of the expert group [39]. The main problem, which was responsible for the relatively large differences among the 29 participating laboratories, was the reference material, as it turned out, which was different in most of the assays used.

In our view, all of the above theoretical considerations play a minor role in laboratory practices when it comes to many of the Lp(a) assays commercially available today. Three important questions must always be answered: 1. Which methods work independently of apo(a) isoforms? 2. Can units be converted from mg/dL to molar concentration? 3. What cut-off values should be applied for risk stratification?

Method

Our preferred commercially available assay is latex-enhanced nephelometry and/or the turbidimetric immunoassay. This stems from the fact that the size of the latex particles compared to Lp(a) is so much greater that the size polymorphism of apo(a) does not matter anymore. Furthermore, in our experience, this method delivers high precision and can be implemented with all standard automated laboratory equipment. ELISA and DELFIA methods are independent of isoforms if monoclonal antibodies are used that recognize only one epitope in apo(a).

Another possibility, which is not yet generally practicable, as there is no harmonized approach yet with respect to routine laboratory practices, is the urine-based analysis of apo(a) fragments. It is mainly fragments with repetitive K-IV that are secreted into urine. Their concentration correlates well with the plasma levels. This method is also at least as effective in differentiating at-risk patients as the plasma-based analysis of Lp(a) [40].

Units

This question is academic to a large extent, since most individuals are heterozygous, i.e. they have two apo(a) isoforms with a very large difference in mass. We, therefore, prefer to state the plasma concentration of Lp(a) in mg/dL, and in our laboratory we use a conversion factor of 3.17, which crystallized from our standardizations: 1 mg/dL apo(a) corresponds to 3.17 nmol/L. This factor also arises when the molecular weight of Lp(a) is assigned a value of

3,150,000, a number confirmed by means of quasielastic light scattering. John Albers, the US expert in the field of Lp(a) analysis, suggests a conversion factor of approx..2.5, which applies to Lp(a) isoforms with a high number of K-IV *repeats* and a molecular weight of 4 million.

Cut-off values

Most studies in recent years have pointed out that Lp(a) is not a "continuous" risk factor, but an increased risk that will only manifest itself once a certain threshold has been reached. Evidence for this thesis is still inadequate, but practical considerations have given rise to cut-off values that, when reached, are supposed to represent an elevated CHD risk. In the first original study that put Lp(a) forward as a risk factor, a cut-off value of 30 mg/dL constituted a relative risk of myocardial infarction of 1.75; a cut-off of 50 mg/dL, 2.3 [14]. These values are very close to those published in subsequent years on the basis of meta-analyses of prospective studies. According to recommendations of the EAS Consensus Report, which are largely based on the Copenhagen Heart Study [4], the cut-off for Lp(a) should be 50 mg/dL, corresponding to about 150 nmol/L.

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