Clinical Lipidology Roundtable Discussion

# JCL Roundtable: Should we treat elevations in Lp(a)?

CrossMark

## W. Virgil Brown, MD*, Patrick M. Moriarty, MD, FNLA, Alan T. Remaley, MD, Sotirios Tsimikas, MD

*Emeritus College, Emory University School of Medicine, Atlanta, GA, USA (Dr Brown); Department of Medicine, University of Kansas Medical Center, Kansas City, KS, USA (Dr Moriarty); Lipoprotein Metabolism Section, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA (Dr Remaley); and Division of Cardiovascular Diseases, Department of Medicine, UCSD Cardiology La Jolla, Perlman Ambulatory Center, La Jolla, CA, USA (Dr Tsimikas)*

**Abstract:** The focus of this Roundtable discussion is the mysterious lipoprotein Lp(a). There is growing evidence that it confers significant risk of vascular disease at high plasma concentrations. The concentration in plasma is highly variable from person to person but relatively stable in any given individual. The issue of defining this as a target of treatment has many facets, which have stymied clinicians in their management of this risk factor. The pertinent questions are many such as: "How does one obtain the most meaningful measure as there are so many components?" "What agents are truly effective in lowering this lipoprotein particle?" "Does direct treatment with reduction affect risk?" "How does low-density lipoprotein–cholesterol relate to the risk?" "If low-density lipoprotein-cholesterol is reduced, is there residual risk related directly to Lp(a)?" and "Are there effective therapies under development?" For this Roundtable, I am fortunate to have three experts that have studied these questions in various settings and have agreed to answer my questions relevant to these clinical issues. These include Dr Moriarty from the University of Kansas, Dr Remaley from the National Institutes of Health, and Dr Tsimikas from the University of California San Diego.
© 2016 National Lipid Association. All rights reserved.

The focus of this Roundtable discussion is the mysterious lipoprotein Lp(a). There is growing evidence that it confers significant risk of vascular disease at high plasma concentrations. The concentration in plasma is highly variable from person to person but given individual. The issue of defining this as a target of treatment has many facets, which have stymied clinicians in their management of this risk factor. The pertinent questions are many such as: How does one obtain the most meaningful

measure as there are so many components; what agents are truly effective in lowering this lipoprotein particle; does direct treatment with reduction affect risk; how does LDL-cholesterol relate to the risk; if LDL-cholesterol is reduced is there residual risk related directly to Lp(a); and are there effective therapies under development? For this Roundtable, I am fortunate to have three experts that have studied these questions in various settings and have agreed to answer my questions relevant to these clinical issues. These include Dr Moriarty from the University of Kansas, Dr Remaley from the National Institutes of Health and Dr Tsimikas from the University of California San Diego.

My first question is for Dr Tsimikas. What is the evidence that elevated Lp(a) is an independent risk factor

* Corresponding author. Emory University Emeritus College, 3208 Habersham Road, NW, Atlanta, GA 30305, USA.
E-mail address: wbrow925@bellsouth.net

for atherosclerotic vascular disease and is this sufficient to establish it as a target for pharmacotherapy?

**Dr Tsimikas:** The answer is yes, I think there are three levels of evidence that we have for establishing Lp(a) as a target for pharmacotherapy. The first level of evidence is the combined epidemiological and meta-analysis data, which probably now encompasses several hundred-thousand patients. In particular, data from the Emerging Risk Factor Collaboration group support that there is a relationship of Lp(a) levels to elevated risk starting at ~25 milligrams per deciliter and increasing linearly as the Lp(a) level rises. This updates and confirms many of the old epidemiologic studies and meta-analyses.

Recent data from the GWAS studies that have linked LPA single nucleotide polymorphisms (SNPS) that are associated high Lp(a) levels to increased risk of coronary disease. This strongly suggests causality from the quantitative measurement of Lp(a) in the plasma to increased cardiovascular risk due to the specific genetic variance.

The third level of evidence is the Mendelian randomization studies, which you can think of as nature's randomized trial in some ways. The studies are not definitive but they are probably as good as you can get short of a randomized trial. For example, you have a group of patients that are born with high Lp(a), and you have a group of patients that are born with low Lp(a), and then, you follow them for outcomes. Because Lp(a) tends to stay stable most of one's lifespan, you can then ask the question, what is the outcome in people that are born with low Lp(a) vs people that are born with high Lp(a). Those studies are all very clear in showing that if you're born with high Lp(a), you're going to have a higher risk of CVD and the risk is proportional to the Lp(a) level.

So, I think those three sets of data point to Lp(a) as an independent and causal risk factor. However, we do not have a randomized trial with a drug that lowers elevated Lp(a) and demonstrates an associated reduction in vascular events.

**Dr Brown:** Do you think LDL lowering is the principle consideration when one discovers a patient with high concentrations of Lp(a)? The related question is: does LDL elevation amplify the impact of Lp(a) on risk?

**Dr Moriarty:** The simple answer is yes. Patients with familial hypercholesterolemia (FH) usually have higher Lp(a) levels compared to the general population. The Lp(a) level is an important predictor of cardiovascular disease (CVD) that adds to the elevated LDL concentration due to specific LDL receptor mutations.

For the non-FH patient even when you lower LDL-C levels with statins, as demonstrated in the JUPITER and 4S trials, those with an elevated Lp(a) had an increased risk of CVD compared to individuals with normal Lp(a) levels.

**Dr Tsimikas:** Yes, there's still residual risk demonstrated in JUPITER when you reduce LDL to quite low values with a statin. Earlier angiographic studies by Greg Brown suggest that if you lower LDL aggressively, the risk of higher Lp(a) in the control group is no longer a contributing factor to disease progression. However, the clinical trial data should cause us to reevaluate that concept.

**Dr Tsimikas:** I think in the JUPITER study, one can evaluate outcomes of Lp(a) since patients achieved values of LDL cholesterol that were well below 70 mg/dL. These are different studies in that Greg Brown's data were in a relatively small cohort and were focused on angiographic measures of the artery, not vascular endpoints. It is hard to compare with the various large statin intervention studies where we have significant changes in clinical endpoints.

**Dr Brown:** The JUPITER trial indicates that reducing LDL-cholesterol as low as 50 to 60 mg/dL may not protect the patient from elevated Lp(a)?

**Dr Tsimikas:** That's right. So, the risk was attenuated by rosuvastatin in patients with high Lp(a) but Lp(a) remained a significant factor in residual risk if elevated. The LIPID trial showed the same thing with pravastatin. AIM-HIGH using niacin and statin therapy had a similar finding. In patients achieving an average LDL-C of 53 mg/dL, if your Lp(a) was in the upper fourth quartile of Lp(a), >~50 milligrams per deciliter, you had an 89% higher risk of subsequent events compared to Lp(a) in first quartile.

**Dr Brown:** In the Aim-High study, niacin therapy did not seem to provide benefit, but there was still a negative effect of Lp(a)?

**Dr Tsimikas:** Yes, I think IMPROVE-IT will be very interesting in this respect, if it explains the substantial residual risk noted in those patients. For example, in IMPROVE-IT, if you look at the absolute numbers, a 34.7% event rate was present in the simvastatin arm and a 32.7% event rate was present in the simvastatin plus ezetimibe arm, so you had only an absolute 2% difference in event rates and mean LDL-C of 54 mg/dL. So, the question is why did 32% of the people come back with a recurrent event if their LDL-C was 54. Were there other risk factors that may have contributed to such a high event rate? I think one is probably Lp(a). There are other candidates of course.

**Dr Brown:** Is there evidence that this risk relationship holds true for stroke and other manifestations of vascular disease similar to coronary artery disease?

**Dr Moriarty:** We have less data on stroke than on CAD. However, we have an interesting anecdote that suggests Lp(a) can contribute to stroke. An 11-year boy, who had experienced multiple stokes, was sent to our institution for removal of a thrombus from his basilar artery. The only abnormality found on workup was an elevated Lp(a) (150 mg/dL) with a normal LDL-C (60 mg/dL). In the young cryptogenic ischemic stroke accounts for 40% of

all strokes and is now believed a good portion of these strokes may be related to Lp(a) levels but unfortunately the marker is rarely measured. Recent studies have determined that children with an elevated Lp(a) level have a 4-fold increase risk of ischemic strokes and children with an elevated Lp(a) have a 10-fold increase risk for a repeat stroke in the first year (reading list). In our case, after the thrombus was removed, we initiated lipoprotein-apheresis to lower his Lp(a) level and risk of another thrombotic event. He has now been on bi-weekly apheresis therapy for 10 months without any CVD events, while his post stroke symptoms have improved.

**Dr Remaley:** It is probably also worth pointing out that a connection between Lp(a) and aortic stenosis has also recently been recognized by Mendelian randomization studies done by Kamstrup et al.

**Dr Tsimikas:** This study confirmed the GWAS study from Thanassoulis et al. published in the NEJM in 2013. Only one genetic variant was associated with aortic calcification, a SNP known as rs10455872, which is the main SNP in individuals of European descent that is associated high Lp(a) levels. In the July 2015 issue of JACC, we examined data from the ASTRONOMER trial, which was a 4-year study of rosuvastatin vs placebo in people that have preexisting mild-moderate aortic stenosis. They asked the question does 40-mg rosuvastatin have an effect on the progression of aortic stenosis and the answer was no. However, patients in the highest tertiles of either Lp(a) or OxPL-apoB had about twice the progression rate of those in the lowest 2 tertiles. The cutoff or the highest tertile was >58.5 milligrams per deciliter for Lp(a). If your Lp(a) was >58.5 mg/dL, your aortic valve disease progressed twice as fast, and you were much more likely to need an aortic valve replacement. So, measuring Lp(a) should be part of the workup for aortic stenosis. Higher levels predict rapid progression to aortic valve replacement.

**Dr Brown:** It would be interesting to analyze samples from the SEAS trial where you have a standardized evaluation of aortic stenosis over almost five years and many lipoprotein measures.

**Dr Tsimikas:** We would love to do that analysis relating the several measures of Lp(a) to the progression of aortic stenosis, particularly as it is a much larger study and has more advanced aortic stenosis. One thing we don't know yet is if Lp(a), and/or OxPL-apoB, is also a predictor in more advanced aortic stenosis where there is a lot more calcification and fibrosis.

**Dr Brown:** Let's turn to treatment of high concentrations of Lp(a). What are the best approaches?

**Dr Remaley:** I'd just like to emphasize what Dr Moriarty said earlier, although we may soon have new drugs that are directed more specifically to lower Lp(a), but the main current therapeutic approach for treating patients with high Lp(a) is to give them a statin. Statins have been shown to reduce cardiovascular risk in patients with high Lp(a), although they have limited effect in lowering Lp(a) itself and presumably are beneficial because they lower LDL-cholesterol.

The other point—which I think we will also discuss later—is even though we all agree that Lp(a) is proatherogenic, it is likely from the FDA's viewpoint that Lp(a) cannot be used as a surrogate for new drug approval like LDL-cholesterol. Perhaps at some point in the future, we can approve drugs based on Lp(a) lowering, but I think the first drugs considered for such approval will be required to show a reduction of clinical events.

**Dr Brown:** It seems that the clinical decision has to be made on the basis of epidemiological evidence at the present time. The studies on the impact of drugs that are designed to lower LDL are minimally effective in reducing Lp(a). This raises a very practical question. When LDL cholesterol is within your goal range what are your Lp(a) goals? What would you do to reach these goals?

**Dr Remaley:** Because of problems with the Lp(a) assay, it makes it somewhat difficult to answer this question, but most of the population studies have shown that there is a definite increase in risk for cardiovascular disease after Lp(a) levels above 30 mg/dL. This is approximately the 70%–75% percentile for the general population other than those of African descent. The advantage of using percentile as your cutpoint is that it is assay independent but requires the user to determine this for each assay and for a representative population. Some people, however, have advocated a cutpoint around the 80% percentile, which is about 50 mg per deciliter for most assays.

**Dr Brown:** What do you think the HERS study contributed to this question? Women who were in HERs were found to have benefit with estrogen therapy if they were in the upper quartile of Lp(a) concentrations. That was defined as Lp(a) level above 27 mg/dL. The estrogen treatment in that group lowered the Lp(a) and seemed to lower the event rate. Has that finding been supported by other studies?

**Dr Remaley:** Well, as you know, Virgil, a lot of people are no longer as enthusiastic about using estrogen replacement therapy for cardiovascular disease prevention based on the Women's Health study and other related studies.

**Dr Brown:** That is certainly the interpretation from the overall data. However, this was a very special group, presumably vulnerable to high Lp(a) and likely to benefit from reduction, making estrogen therapy a reasonable choice?

**Dr Tsimikas:** We know that postmenopausal women, as they lose their estrogen may have a rise in Lp(a) levels up to 30%. So that goes along with the data on estrogen

**Dr Remaley**

**Dr Brown**

replacement where the Lp(a) levels go down and in post hoc analysis patients seem to do better. So, it is hypothesis-generating data because it wasn't a randomized trial by Lp(a) levels.

But then what other data do we have? We have all the apheresis data—which Patrick can comment on. There have been about five studies showing dramatic reduction in events when people go on apheresis for high Lp(a). So, I think if you look at evidence for Lp(a) reduction and risk, short of a randomized trial, HERS and all the apheresis studies are probably the two best sets of data that we have at this point that have demonstrated evidence of potential therapeutic benefit.

**Dr Brown:** Is there a threshold value that you believe justifies treating Lp(a) in the presence of desirable LDL cholesterol values?

**Dr Tsimikas:** I can tell you what I do in my own personal practice. I'm a cardiologist so I see a lot of patients with signs of CAD and in need of diagnostic evaluation. I check Lp(a) on everybody in my clinic at least once. Most of these are secondary prevention patients, so that's within the consensus statements from EAS and NLA. Interestingly, when I last checked, 49% of my patients had an Lp(a) level of >30 milligrams per deciliter and about 35% >50 milligrams per deciliter. I have developed a dedicated "Lp(a) clinic" beginning in 2014. There, I only see high Lp(a) patients. My practice also has a lot of very young patients in this clinic.

**Dr Remaley:** So, there is clearly an enrichment of patients with elevated Lp(a) in your regular clinic because you wouldn't expect those kind of numbers based on the general population distribution of Lp(a).

**Dr Tsimikas:** Right, exactly. In managing those with elevated Lp(a), I first get the LDL-C as low as possible, aiming for 50 mg/dL. I greatly emphasize this in those who show advancing disease or those that are very young with disease. In dealing specifically with the Lp(a) issue, I use niacin quite a bit. Many patients will tolerate niacin but you must warm them of the adverse effects, particularly the flushing reaction. If a patient can tolerate niacin for about a year, they generally do well thereafter. I monitor glucose and uric acid but significant elevations in these are relatively uncommon. It is known that niacin can raise glucose ∼6 mg/dL, so this is something to watch closely in metabolic syndrome patients.

Admittedly, it is the art of medicine at this point, using the data we have discussed for support. Ultimately, we need the intervention study to stimulate a wider consideration of treating Lp(a).

**Dr Brown:** You are a student of this problem and with all your experience, you make a clinical judgment to be aggressive in many of these patients and you lower Lp(a). Now in your general clinic, after evaluating risk and controlling LDL concentrations, do you have a range of values that would cause you to focus further treatment on Lp(a) reduction?

**Dr Tsimikas:** If their Lp(a) >50 mg/dL, I make a consistent effort to lower this value. Of course, those with active vascular disease and values >50 get my special attention in this respect. It is again of note that 43% of our cardiology patients had values of Lp(a) >50 mg/dL.

**Dr Moriarty:** This is from the whole hospital?

**Dr Tsimikas:** Yeah, the whole hospital. I'm getting a lot of referrals from the cath laboratory. Occasionally you, you uncover a surprising patient such as a recent 43 year old with STEMI and an Lp(a) of 240 mg/dL. If you do not check, you miss the diagnosis in such patients.

**Dr Remaley:** Do you consider adding aspirin or carnitine in these patients?

**Dr Tsimikas:** Yes.

**Dr Remaley:** Maybe in kids with this, where aspirin might be useful.

**Dr Moriarty:** Well, if they're secondary prevention, then they should all be placed on aspirin.

**Dr Tsimikas:** The patients I see, I do see some high-risk primary prevention. I usually put them on aspirin and there are some nice data from the Women's Health Study from Paul Ridker where they looked at a retrospective analysis in ∼26,000 women in this aspirin trial. The whole overall trial was negative but the group of women that benefited was the ones that had Lp(a) that were in the fourth quartile. So, women with high Lp(a) seem to do better with aspirin. Again, this is hypothesis generating, but if it is a low bleeding risk, aspirin is always part of my treatment in patients with high Lp(a).

**Dr Remaley:** And Carnitine?

**Dr Tsimikas:** I have not used Carnitine. I have seen some of the data suggesting it may lower Lp(a) levels.

**Dr Brown:** The debate as to whether the relationship between atherosclerosis and Lp(a) is due to thrombosis or to direct causality in lesion development continues. A response to aspirin would certainly suggest that might be the case. However, if lesions heal and endothelium becomes healthier under the influence of statin, would you expect the thrombotic issues to be much less and Lp(a) related risk to decline?

**Dr Tsimikas:** The thrombotic issue is quite interesting. In CAD, you cannot separate arterial thrombosis from atherosclerosis. In venous thrombosis, there have been two studies, both in terms of gene variants of Lp(a). In the Copenhagen Heart Study, Lp(a) levels do not seem to associate with venous thrombosis. This was a large study, which included >40,000 persons. The study other one was from Iceland that evaluated LPA SNPs, it showed an association with MI but not with venous thrombosis. So, the question is do you need to have atherosclerosis for thrombosis to be a risk factor.

**Dr Brown:** Yet, there is evidence from the JUPITER trial that venous thrombosis is reduced with statins. So, it's complicated.

Now I would like Dr Remaley to discuss the genetic control of Lp(a) concentrations, Alan? What are the basic issues?

**Dr Remaley:** First of all, unlike LDL-cholesterol and HDL-cholesterol, which is about 50/50 in terms of the genetic contribution vs environment, genetics play a much bigger role in determining Lp(a) levels. I think the best estimate is that genetic variation accounts for about 90% of Lp(a) levels. This is the main reason you don't need to measure it too often, although that may change in the future once we have more specific and effective therapies for lowering Lp(a). There are probably multiple genetic factors that contribute to Lp(a) levels, but the one we understand best is the number of kringle repeats, particularly the kringle-IV, type 2 isoform. There are two relatively common SNPs in this locus that accounts for about 30%–40% of the variation of Lp(a) levels. There are also some SNPs in the promoter for Lp(a) that can affect its level. The other thing that is interesting from a protein chemistry standpoint is these kringles, which contain about 100 amino acids, have three disulfide bonds. I have a theory as to how structure of Lp(a) may relate to plasma concentrations. In general, it is difficult during protein secretion to properly form disulfide bonds, and in general proteins with a lot of disulfide bonds do not fold as efficiently or a quickly and as a consequence are often poorly secreted. So, in this theory, large apoA isoforms, which have many kringles, sometimes as many as 100, are inefficiently secreted. This may account for the inverse relationship between serum Lp(a) levels and the presence of apo(a) genes coding for a large number of kringles. The rate of catabolism of the different size isoforms and their rate of association with apoB in the plasma compartment are also potential issues of importance in determining plasma concentrations. However, the main driver for Lp(a) plasma levels is probably due to the differential secretion of the different size isoforms.

**Dr Brown:** So, the shorter chain, smaller Lp(a) have a higher plasma concentration in terms of the number of particles. Although they're smaller, these are the structures that are most clearly related to atherosclerosis. I assume that is because there are so many more of them per unit volume of plasma?

**Dr Remaley:** That and maybe because of their smaller size. The idea is similar to the concept of how small LDL is more proatherogneic. Smaller Lp(a) particles may also infiltrate better into the vessel wall where they can initiate atherosclerosis. There is also some data that suggest that the extracellular assembly of apo(a) is important and that once the smaller apo(a) dissociated from apoB, they can more readily reattach to apoB by a disulfide bond, and hence, the levels of smaller Lp(a) isoforms are better maintained.

**Dr Brown:** Have we now refined and standardized the assay for Lp(a) that best defines its relationship to risk of vascular disease?

**Dr Remaley:** I think we are getting there, but there is still work to be done. Part of the controversy about the relevance of Lp(a) to cardiovascular disease relates to problems with the assay. I remember in the mid-1980s there was much enthusiasm for it as a positive risk factor for cardiovascular disease and then this enthusiasm dimmed in the 1990s. There were several studies at the time, such as the Physician Health Study that failed to show a strong association of Lp(a), but a likely cause for some of these negative studies was problems with the assay.

There are about 40 assays that are approved by the Food and Drug Administration (FDA) for measuring Lp(a), and the main metric is Lp(a) mass. This causes a lot of confusion, as Lp(a) mass means the whole particle and includes both lipid and protein. Most methods depend on the reaction of antibodies with apo(a) protein and the formation of an immune complex, which are measured by turbidity or by a nephelometer (McConnel in Reading List). Unfortunately, many of the first anti-apo(a) antibodies developed react with several of the repeat kringle units, which creates a positive bias for the larger isoforms with more kringles and a negative bias for the smaller size isoforms with less kringles. There has been great effort going back now more than 20 years to better standardize the Lp(a) assay and much of this has been led by Dr Marcovina. It is still challenging problem, but I am encouraged that there is greater awareness of the problem and some major diagnostic companies have now produced assays that are isoform independent.

Another problems is that until recently, because of the relatively low demand for Lp(a) testing, most assays were based on ELISAs that have less precision and are not commonly done by many clinical laboratories. There is now at least one fully automated Lp(a) assay by Roche, and as it becomes more widely adopted, we will have more precise and at least better harmonization of Lp(a) testing and perhaps more accurate results as well.

**Dr Brown:** So, these are nephelometric assays that are rapid, inexpensive, and well standardized?

**Dr Remaley:** A recent advance in this area was the development of a program by the College of American Pathology (CAP) called the accuracy-based lipid program. The CDC, for a long time, has also had a program for standardizing lipid and lipoprotein testing but never focused on Lp(a). All clinical labs are required to do what is called proficiency testing to assess the accuracy of their test results. They receive unknown samples several times a year by the CAP or some other organization, and their results are compared to a peer group that use the same assay. The CAP now produces frozen serum pools for assessing accuracy of lipid and lipoprotein tests, including Lp(a). It has been shown that inaccurate Lp(a) assays with the negative bias for the small isoforms can misclassify as many as 10%–15% with high Lp(a) that should probably be classified as a lower risk category.

**Dr Tsimikas:** One of the major issues of the commercial labs is that they use polyclonal antibodies, unlike the Marcovina assay, which uses monoclonal antibodies. Most commercial assays are actually not isoform independent because all the polyclonal antibodies will bind multiple sites on the KIV-2 repeats. The way these assays are made close to being isoform independent is with the

calibrators. However, because the calibrators are derived from pools of plasma, you have to retest it with each new batch. Reporting values as mass of Lp(a), ie, mg/dL, measures the entire mass of the particle, including apo(a), apoB, cholesterol, triglyceride, phospholipids and carbohydrates, and the relative values of each varies among patients. So, I agree with you that there are too many assays, they have to be standardized, everybody has to move to reporting in terms of particle number like nanomoles per liter.

**Dr Remaley:** I agree, but it might be premature to switch to nanomoles per liter until we know the assays are accurate. As you know there has been some debate about what conversion factor to use. Historically, it was around 2.7 but now people are recommending a slightly higher number, and different manufacturers use different conversion factors, which contribute to the confusion about Lp(a) assays.

**Dr Tsimikas:** What I'm suggesting is that the calibrators now have to be reported in nanomoles per liter measured with assays that signal particle number. In that way, you never have to use a conversion factor and you get the raw data as it should be.

**Dr Remaley:** I agree but a common misperception is that because an assay is reported in nanomoles per liter that it must be accurate and isoform independent, which is not necessarily true.

**Dr Brown:** In the clinic, this misunderstanding of the values received and their relationship to the literature could lead to very inappropriate treatment. On the other hand, this could be a situation where perfect is the enemy of good. The variance in Lp(a) concentrations defines a wide range of value that are acceptable. If the assay is off about 10 or 15 units, the result may impact few decisions. However, not appreciating that different units of measure are reported could cause a very large error.

**Dr Tsimikas:** That's a very good point. In general, if you were over 100 milligrams per deciliter or an equivalent measure, the unit you use doesn't matter. It's only important if we're classifying patients as above normal or below normal.

**Dr Remaley:** Yes, I agree too that the assay problems are most relevant for results near the cut point.

**Dr Tsimikas:** But at the clinical level though, if you have a high level, you'll have a high result on all the assays.

**Dr Brown:** And if you have a very low level, say 10 mg/dL?

**Dr Tsimikas:** You won't have to check it again. You only have to check it once in these patients.

**Dr Brown:** Exactly. So, I think that's an important point we want to make that we're working hard to get a great assay but we can still use what we have to make important clinical decisions. Of course this will gain in importance once we have strong evidence supporting treatment and the availability of more effective treatment.

**Dr Moriarty:** In our clinic we see patients with extremely high Lp(a) who have absolutely no vascular history of disease or patients with modestly-elevated Lp(a)'s and CVD. We have used labs, which measure Lp(a) mass, cholesterol content and particle #.No matter what assay is used if there is no underlying history of premature CVD, family history or FH we will generally not treat the Lp(a). In regards to assays, Dr. Tsimikas and his colleagues have developed an oxidized phospholipid bound Lp(a) assay to determine its association to cardiovascular risk. Is this the answer of why Lp(a) causes vascular disease? I don't know. It may be some other inflammatory or thrombotic marker? I think the jury is still out, but as I stated if the number is high without underlying risk, I'm a little bit leery of just going after it without additional evidence of risk.

**Dr Brown:** I think that a lot of people don't realize though that the curves are so skewed and strange with Lp(a) that a significant majority of people are going to have a very acceptable number; it's only going to be perhaps the upper 20, 25% that we really need to be concerned about. If you are monitoring a cardiology clinic the values will be quite different as we have heard.

**Dr Tsimikas:** Currently, making sure that it is measured at least once is much more important than the accuracy of the assay used.

**Dr Moriarty:** Yes, I agree.

**Dr Tsimikas:** One fact worth mentioning about the Lp(a) assays that depend entirely on some estimate of the cholesterol content have not been validated for prediction of outcomes. I don't think people realize that this has been studied in the Framingham Heart study and Lp(a) cholesterol assay did not predict outcomes but a strong relationship to events was reported with Lp(a) mass assays. So, I just want to caution people when they look at purported cholesterol content of Lp(a) alone the database for predicting outcomes is not there yet.

**Dr Remaley:** There is also an electrophoresis assay for measuring the cholesterol content of Lp(a) also by Health Diagnostic Laboratory but there is only one study - by Dr. Joe McConnell showing its positive association with cardiovascular disease by an imaging study.

**Dr Tsimikas:** We just need a stronger database for this assay as well.

**Dr Brown:** I would like to return to potential mechanisms of atherogenesis. There are at least three postulated mechanisms of Lp(a) for a role in lesion development. One is that it just comes into the artery wall and is metabolized abnormally because it doesn't interact with the LDL receptor, and so it's going to be bound and perhaps degraded and enter the macrophage in a way that leads to cholesterol accumulation. The second is that it has a thrombotic effect because of its interaction with fibrin and plasminogen, preventing degradation of fibrin by activated plasmin. The third and most recent is that it's interestingly binding to and accumulating oxidized lipids, which stimulate abnormal cellular metabolism when they enter the artery wall. Do you think all of those are playing a role?

**Dr Tsimikas:** If you look at all the bad things Lp(a) does, you should recognize that the LDL moiety of Lp(a)

contributes everything a normal LDL does. The other question is what does the apo(a) component contribute. The apo(a) component contributes oxidized phospholipids, which are covertly bound to apo(a), but interestingly, we also find that there are extractable oxidized phospholipids from the lipid phase of the LDL component. We have just published with Dr Koshinsky's group in the Journal of Lipid Research showing that the oxidized phospholipid component on the apo(a) upregulates IL-8 secretion by macrophages. Last year, we published data that the apo(a) component binds MCP-1. There has been a whole series of papers showing pro-inflammatory effects of the apo(a) molecule. The kringle-IV type 2 repeats, however, are relatively inert biologically. They mediate the high levels of Lp(a) but there's not a lot of cellular biology associated with them. Most of the biological effect of apo(a) is at the other end.

The lysine binding sites on kringle-IV-10 actually will bind to exposed lysine on endothelium or the vessel wall once Lp(a) gets in and won't come off very easily, even with detergents. So, Lp(a) is very sticky, much more than LDL. So, when it gets into the proteoglycan matrix, it'll stick there, deliver the cholesterol and the oxidized phospholipids to monocyte macrophages, and induce inflammation. Nobody has been able to quantitatively separate all the different mechanisms of risk.

When we developed the oxidized phospholipid per apoB assay (OxPL-apoB) we expected a strong correlation with LDL but we instead found a strong correlation with Lp(a). In every study that we have published, if we measure OxPL-apoB vs Lp(a), OxPL-apoB is either similar in predicting risk or superior; it's never been inferior. So, we think that the key driver of the inflammation that's associated with Lp(a), such as macrophage activation, IL-8 and MCP-1 secretion is due to the OxPL. I don't think it's the only driver but it certainly seems to be a major component, supported by at least 40 published papers in a variety of clinical cohorts.

**Dr Brown:** What is the effect of loading oxidized phospholipids on to the apo(a) protein and its effect on thrombogenesis?

**Dr Tsimikas:** That effect Ox OxPL on thombogenesis has not been studied yet. We haven't had a chance to do that, but I just want to mention the oxidized phospholipids are present attached to one other circulating protein, and that's plasminogen. In fact, there are more oxidized phospholipids on plasminogen than on Lp(a).

**Dr Remaley:** On the kringles as well?

**Dr Tsimikas:** Yes, we believe in the homologous sites to apo(a).

We haven't identified exactly, which amino acids on apo(a) or plasminogen bind oxidized phospholipids, but we know that the lysine binding pocket on kringle IV-10 is crucial for binding, because if you mutate that pocket you no longer can find immunoactivity for oxidized phospholipids on the Lp(a).

**Dr Brown:** Is this oxidized phospholipid, is it mainly phosphatidylcholine with an oxidized fatty acid in the two position on the glycerol backbone?

**Dr Tsimikas:** Yes, when that sn2 fatty acid is truncated by oxidation it generates, among other things, an aldehyde or a ketone that will then bind with a lysine or an arginine or a histidine on the protein, and generate a complex adduct.

**Dr Brown:** Are the oxidized derivatives derived predominately from phosphatidylcholine?

**Dr Tsimikas:** We haven't looked for other non-PC based, but we can find a variety of oxidized phospholipids on Lp(a), some of them that are recognized by antibody E06 but some that are not, but they're all phosphocholine based. We have to broaden this and look for other kinds of non-PC based phospholipids.

But to get back to plasminogen, I just want to make one more point because it gives you a different pathophysiology if the OxPL was on plasminogen vs if it's on Lp(a). So, in Lp(a) we clearly have shown it predicts cardiovascular events and is pro-atherogenic. When the oxidized phospholipids are on plasminogen, it actually potentiates fibrinolysis, meaning that tPA can cleave the plasminogen to plasmin faster and it works better. If you remove the oxidized phospholipids from plasminogen, fibrinolysis is delayed. So, you have a ying-yang here of plasminogen vs Lp(a). In one context, when OxPL are on Lp(a), they are bad guys; when it's on plasminogen, it seems to be a good guy. We have to do a lot more work on plasminogen to understand the clinical implications.

Maybe that's one part of the whole regulatory pathway of fibronolysis. If you're not making any clots, Lp(a) probably doesn't do anything. When you start making clots, Lp(a) can potentiate progression and prevent them from being lysed. It is an anti-fibrinolytic pathway. So, you have to have a reason to cause a thrombus to begin and then it's a bad guy. If you're not making thrombi, there may be no deleterious effect. Although 25% of the population has high Lp(a). We're not seeing 25% of people coming with thrombotic events.

**Dr Brown:** Are you suggesting that this is because they don't have damaged vascular surfaces that set the stage for the thrombosis.

**Dr Tsimikas:** Exactly. So, it would have to be a second hit phenomena.

**Dr Moriarty:** Do you believe the Lp(a) itself can be the mediator of the vascular disease first?

**Dr Tsimikas:** That's another question, yes. I think clearly it is. The data relate primarily to the risk for MI.

**Dr Moriarty:** And why does it occur for some and not others.

**Dr Tsimikas:** So, you have to have, I think, a second hit, which goes back to having high LDL or smoking or having some vascular disease where it becomes a really bad guy.

**Dr Remaley:** Are there any studies showing sequential changes in the level of oxidized phospholipids bound to plasminogen during clot formation, followed later by enrichment on Lp(a)? If so, it may suggest that Lp(a) could antagonize plasminogen function by sequestering oxidized lipids.

**Dr Tsimikas:** We published a study recently in which we followed patients with acute coronary syndromes and

measured temporal changes in oxPL on plasminogen and on Lp(a). During this acute coronary syndrome, the oxPL on Lp(a) goes up and the oxPL on plasminogen goes down. This would be consistent with promoting a thrombotic event. That is the only data we have, but it wasn't linked to future outcomes. It was only a study of only about 3 months.

**Dr Brown:** What about children with sickle cell disease? Do they have issues with Lp(a)? Does high Lp(a) concentrations accentuate the chances of having a stroke in those kids?

**Dr Tsimikas:** That's a great question. I'm not aware of anybody looking at that. It would be a great study to do actually.

**Dr Moriarty:** That's more related to the aggregation of the blood from the sickle, I think, than to coagulation cascade.

**Dr Brown:** Not every kid with sickle cell disease has a major problem with thrombosis. Only some of them have a terrible problem with it.

**Dr Moriarty:** The boy I mentioned earlier with an elevated Lp(a) and multiple strokes was found to have a small tear on the basilar artery where the thrombus developed. The radiologists who performed the examination claimed the tear was too minuscule in size to be the source of his CV events.

**Dr Brown:** Perhaps that fits with the idea that Lp(a) is an accelerant, not a primary cause of stroke.

I would like now to turn to treatment and their potential value in patients with high Lp(a). Is there any convincing evidence that niacin may offer some value in reducing Lp(a) with regard to reducing vascular events?

**Dr Tsimikas:** I can comment on the AIM-HIGH. So, AIM-HIGH looked at this obviously and on niacin, it had no effect on outcomes. It lowered Lp(a) about 24% but keep in mind that the entry level there for Lp(a) was the equivalent of $\sim$13 milligrams per deciliter.

However, the fourth quartile had values $\sim$125 nmol/L, equivalent to >50 mg/dL. So, a quarter of the patients, 25%, would be considered to have high Lp(a).

**Dr Moriarty:** The higher their Lp(a) was, the higher the event rate despite a lowering of Lp(a) by >30% with niacin.

**Dr Tsimikas:** So, if you take a patient that has stable CAD with an LDL of 55 and you lower a normal level of the Lp(a) 30%, it doesn't look like there is a benefit. However, AIM-HIGH was underpowered to address this issue.

We would need a niacin trial limited to patients with high Lp(a). We probably won't ever see that now but that could have been a trial to answer your question.

**Dr Moriarty:** HPS2-THRIVE, using extended-release niacin-laropiprant, hasn't published their data yet but as they measured Lp(a) in a very small subset, I don't think they're going to be able to answer this question.

**Dr Brown:** Several of the new drugs recently reviewed for prescription use are effective in reducing Lp(a). What are the effects with the antisense oligonucleotide that lowers apoB (mipomersen), the microsomal triglyceride

transport inhibitor (lomitapide) or the two PCSK9 inhibitors, alirocumab and evolocumab? How much information do we have about treating people with very high Lp(a) with these particular drugs?

**Dr Moriarty:** As of yet, there are no reports with such subgroups of high Lp(a). The MTP inhibitors have a very limited effect on Lp(a), and this effect seems to be transitory. Mipomersen is presently the most potent drug we have in lowering Lp(a), which is similar to its range in the reduction of LDL (10%–80%) with an average reduction around 30%. The PCSK9 inhibitors reduce Lp(a) by about 20%–25%.

The PCSK9 inhibitor outcome trials (Fournier, ODYSSEY and SPIRE) will have some information on Lp(a) as a secondary endpoint that's 27,000 in the Fourier trial with evolocumab, 18,000 in the ODYSSEY trail with alirocumab, and 26,000 in the SPIRE trail with bococizumab. That is over 70,000 patients in these trials with a good percentage of them probably having an elevated Lp(a).

**Dr Tsimikas:** All these drugs—niacin, mipomersen, PCSK9 inhibitors seem to have a ceiling of Lp(a) lowering, and I'm not really sure why but you're getting 60% reduction in LDL with PCSK9 but you're only getting 20%–30% reduction in Lp(a), so there's some disconnect there.

**Dr Brown:** I am puzzled by the reduction in Lp(a) with drugs that are believed to ultimately act by increasing LDL receptor number yet statins, which also act through the same mechanism have little or no effect on Lp(a). How do you explain this?

**Dr Tsimikas:** We're looking at this in our laboratory now with PCSK9 antibodies and it's probably not reduction in the synthesis of apo(a). It could be assembly or it could be clearance. It doesn't look like there's a direct effect on apo(a) transcription. The mechanisms are not defined yet. It's probably some clearance mechanism—I'm just hypothesizing here—and it's probably not just one mechanism but probably multiple ones. Dr Remaley did some studies showing that the SRB1 receptor is involved in Lp(a) clearance. Plasminogen receptors and other unknown receptors may be involved.

**Dr Remaley:** You need the apoB to form Lp(a), but you usually have excess apoB. If you have drug, however that is very efficient in lowering apoB, you will also impact on Lp(a) assembly.

**Dr Brown:** Are all the pertinent questions about Lp(a) assembly now answered? Does the addition of apo(a) occur only in the plasma compartment, not in the liver? What are the requirements of an apoB containing lipoprotein to be able to bind to apo(a) and form a covalent bond?

**Dr Tsimikas:** I think it's interesting because the liver makes a lot of the VLDL, but there's very little apo(a) on VLDL. You find that when people are hypertriglyceridemic, maybe 5% of the apo(a) mass is on VLDL. Perhaps, the liver is secreting a population of LDL-sized particles that can form Lp(a) in the golgi or endoplasmic reticulum?

**Dr Brown:** Perhaps, it is synthesized in the space of Disse with circulating LDL that has derived from VLDL?

**Dr Tsimikas:** Right. So, there's two possibilities. That's a second one.

**Dr Brown:** And we know that remnants get converted to LDL in the space of Disse, so there's hepatic lipase action that may be involved. Hepatic lipase is a very active enzyme and one of the major things it does is the conversion of VLDL molecules into LDL molecules by removing triglycerides and other components. It would seem that after most of the triglyceride is removed, the apoB could present itself in a new format allowing a better presentation of apoB for apo(a) binding? Is that a possibility?

**Dr Remaley:** I think what we understand about it chemically is that the location of the cysteine involved in the disulfide bond with apo(a) is near the C-terminus of apoB.

**Dr Tsimikas:** It's very near the LDL receptor binding side of apoB.

**Dr Remaley:** Presumably, the kringles that bind lysine recognize lysines on apoB, but the exact residues have not been described.

**Dr Brown:** We know apoB is this huge polypeptide chain stretched out over a big VLDL particle and that it must change its configuration as the triglyceride is removed and the size shrinks. The presentation to the aqueous medium of different loci on apoB must change as well.

**Dr Remaley:** Several groups have tried to use N-acetyl-cysteine to reduce the disulfide bond between apoB and apo(a), and there was some early promising results, but subsequently, it has not been shown to be a practical or effective way to lower Lp(a). Likewise aminocaproic acid, a lysine analog, which is used clinically to inhibit fibrinolysis by plasminogen, can also partially lower Lp(a), probably by interfering with the association of apo(a) with apoB by the lysine groups. A better understanding of the structure of Lp(a) could lead to new strategies in drug development.

**Dr Tsimikas:** What's interesting epidemiologically is that there's an inverse correlation between Lp(a) and triglycerides; the R value is ~0.2. This was published from data in the MIRACL trial, and there are several large studies that found similar relationships. Patients with diabetes who tend to have high triglycerides tend to also have lower Lp(a) levels. So, there's clearly some kind of differing presentation of VLDL in the formation of Lp(a).

**Dr Brown:** This is clearly complicated and in need of testable hypotheses.

**Dr Tsimikas:** We have noticed in our data we get about a 10% to 20% increase in Lp(a) with statins. We just published an article with rosuvastatin in aortic stenosis in JACC recently where the Lp(a) level went from 45 to 55 milligrams per deciliter with rosuvastatin. This increase can be quite dramatic. I'll give you two examples of patients in our clinic in their 40s with high Lp(a) and strong family histories of CAD. One with a pretreatment value for Lp(a) of 143, after 1 month of atorvastatin 20 mg/day, the value for Lp(a) was 206 mg/dL. The second patient had a baseline value for Lp(a) of 106 mg/dL. After treatment with atorvastatin 20 mg/day, it went up to 160 mg/dL. He found that he could not tolerate the statin so I took him of it, and

the Lp(a) fell to about 95 mg/dL. So, I've noticed this epidemiologically that a certain number of patients, particularly with a high Lp(a) tend to go even higher when you put them on statins. I don't know what impact this has, but in 4S, going way back now in the 90s, the patients that had a high Lp(a) were the least to benefit from simvastatin.

**Dr Brown:** In those cases, as you mentioned, did you look at their oxidized phospholipids?

**Dr Tsimikas:** No, we didn't do that.

**Dr Brown:** But you can see the statins raise that also.

**Dr Tsimikas:** They do actually. In fact, we have an article on this topic that was just accepted in *Journal of Clinical Lipidology*. As Lp(a) is the main lipoprotein OxPL carrier, the OxPL-apoB changes in the same direction as Lp(a).

**Dr. Brown:** I am aware that lipoprotein apheresis lowers Lp(a) and this has been in studies. Dr Moriarty has extensive experience with this therapy. Are there data relating measures of aortic stenosis to changes in Lp(a) with apheresis?

**Dr Moriarty:** A recent article in atherosclerosis suggested the initiation of Lipoprotein-apheresis before the onset of aortic root atheroma should reduce the requirement for aortic surgery in HoFH patients. The authors did not specifically measure Lp(a) but patients with HoFH usually have elevated Lp(a) levels.

**Dr Brown:** Could you tell us more about the studies with apheresis and the role of apheresis in managing very high Lp(a)?

**Dr Moriarty:** The treatment of an elevated Lp(a) with apheresis started in Germany. Three observational prospective and/or retrospective trials have been published. The trials involved over 300 patients with a mean Lp(a) and LDL-C of about 100 mg/dL. They all had CAD and about 90% were on statin therapy. The primary endpoint was major atherosclerotic cardiovascular events (MACE) as measured 2–5 years before and after initiating apheresis. All three trials demonstrated a 70%–90% reduction in the incidence of MACE. Based on these trials, and the unwillingness of physicians to perform a placebo controlled trial, the German government approved apheresis for patients with ongoing CVD and Lp(a) levels >60 mg/dL irrespective of LDL-C levels.

It's powerful data, but the question persists as to whether the event reduction is attributable solely related to Lp(a) removal or is it in addition to the reduction of LDL-C, inflammatory markers, and improved blood rheology?

One study of Russia attempted to answer this question. They used an apheresis device, which removes only Lp(a) and not LDL-C due to special columns containing antibodies to Lp(a). Their study, which was published in atherosclerosis, examined changes to atheroma volume in the coronary arteries on patients with CAD. Thirty patients were divided in two groups: statins or statins plus weekly Lp(a)-apheresis. In both groups baseline Lp(a) was about 100 mg/dL and LDL-C 80 mg/dL. After 18 months of

treatment, the apheresis group had >20% reduction in atheroma volume compared to no change in the statin only group.

**Dr Brown:** Most of these studies have used historical controls or other experience. Have any used true random control assignment?

**Dr Moriarty:** In the Russian Lp(a)-apheresis study, they had a control group receiving only atorvastatin 40 mg/day. The German trials did not use separate control groups but instead compared the patients cardiovascular history before and after initiating apheresis. As I mentioned earlier, the German government did propose a placebo controlled study for these patients with an elevated Lp(a) but most of the apheresis sites refused to participate in the trial based on their belief that it would be unethical not to treat these patients with apheresis.

**Dr Brown:** Does a standard apheresis with a dextran sulfate column remove as much Lp(a) on a percentage basis as LDL?

**Dr Moriarty:** Just about the same. And the Braun apheresis device (HELP, Futura), which uses heparin instead of dextran sulfate to remove the apoB lipoproteins, also lowers Lp(a) and LDL-C by a similar amount. A nice aspect associated with the Kaneka machine is that you are able to treat more plasma volume where the Braun device can only treat about 3 liters of plasma.

**Dr Brown:** My concern is that if we don't do a well-controlled trial, people will never accept the data as being evidence based. Do you know of any plans to do a randomized controlled trial?

**Dr Moriarty:** As the treatment of Lp(a) with Lipoprotein-apheresis has already been approved by the German government, I doubt you will see this type of study performed in their country. Presently, they regularly treat about 2000 patients with apheresis for dyslipidemia and about 1200 are specifically for Lp(a) removal. The most appropriate trial to demonstrate the benefit of reducing Lp(a) with apheresis should involve the Russian Lp(a)-apheresis machine but I doubt they will ever receive the necessary funding to perform an outcome trial.

**Dr Brown:** The lack of another effective treatment provides a rationale. Unfortunately, their message may be very slowly accepted without a better science base for effectiveness. Perhaps that is the major block to a truly convincing study?

**Dr Moriarty:** For the patients themselves; it is convincing. Two or 3 years before initiating apheresis, they had progressive cardiovascular disease events per year, and after initiating apheresis, their events continued to decline. If you graphed out the event rates, it would represent a pyramid with the peak right before initiating apheresis.

**Dr Tsimikas:** By definition, it has to go down because these people are getting bypassed and getting stents, so their event rate may go down because of other therapy. The question is how much lower does it go by doing apheresis vs the application of modern medical care?

**Dr Brown:** And without a control group, there will always be argument about such data. Will these data always be questioned as valid?

**Dr Tsimikas:** There's one other thing worth mentioning with apheresis. The absolute reduction postprocedure is very potent but Lp(a) comes back another ∼10 mg/dL or so every day. So, by the time you go back to your next visit, you're basically up to your baseline. So, if you do the time average reduction—what would you say, Patrick, 35%, 40% at most?

**Dr Moriarty:** Yes, 30% to, 40%.

**Dr Tsimikas:** So, it's only modestly more effective than niacin?

**Dr Moriarty:** But unlike niacin, apheresis also lowers LDL-C by 70% and a multitude of other proatherogenic proteins that probably play an important part in the CVD event reduction.

**Dr Brown:** Most of the benefit seems obvious when you follow such patients but my fear is that even with a series of patients who have apparent success, we cannot convince the third party payers to back this in significant numbers. I think we all agree that we need a pharmaceutical approach that provides a very large reduction in Lp(a) and that needs to be tested in patients with values >50 mg/dL.

I want to thank each of you for a vigorous discussion of an area that has stimulated rapidly developing interest among lipidologists. We need more research into the mechanisms of synthesis, clearance and its relationship to arteriosclerosis. The epidemiology and genetics of Lp(a) are indicating that this will ultimately be a valuable target for treatment. However, until we have a method of treatment that is very effective in lowering plasma concentrations and use that in controlled clinical trials to demonstrate reduction in vascular events, the wide spread interest in treatment will not develop to its potential.

## Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jacl.2016.02.012.