January 2012 Issue | Jake Orville, President & CEO

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Welcome to *Functional Medicine Update* for January 2012. Can you believe it, another new year for *Functional Medicine Update*? I have to say, of all the things that I've done over the years, one of the things that rises to the surface is *Functional Medicine Update*. To think that we're now at more than three decades of our activity with regard to this audio magazine. What I would say to all of you is that this has been an extraordinary run of learning.

Cardiovascular Extended Risk Factor Assessment

This month my introduction is on cardiovascular extended risk factor assessment. The reason that cardiovascular seems noteworthy is, as you know, it's the number one cause of death (still) in much of the world, certainly in the developed world it's true (developed countries). Secondly, it's because this area of extended risk factors, or biomarkers, really relates to the very fundamental question of function, because rather than looking at overt pathology, these evaluative tools that we call biomarkers are really looking at aspects of disturbed physiological function or disturbed pathology. One might really view a biomarker in two respective ways. One is you might look at a biomarker that is analyzing the primary agencies that cause a specific dysfunction and urge, then, or contribute to, the development of an ultimate disease. And the second is a biomarker that looks at secondary effects of that disease process; it's kind of the smoke that comes from the fire that tells us how severe the insipient disturbed metabolism or pathology is at that state in the patient's life cycle.

Cholesterol is a Biomarker, Not the Cause of Disease

So you can really look at two different kinds of biomarkers. Let's use cholesterol as an example. I don't think that we believe that the elevation of blood cholesterol isn't, of itself, the necessary pathognomic agent that causes disease, but it's a marker for a state of disturbed metabolism that is associated with disease, and therefore we would call this kind of the precedent landscape that sets the tone for a disease called atheroma. We would look at things like phospholipase A2 (PLA2), which is a measurement of the inflammatory burden from a disturbed plaque: here we're looking at the smoke that comes from a fire, and how it interrelates, then, to the progression of a particular pathology that will become a disease.

As we examine different biomarkers, we can ask where they fit into this sequential series of events moving from optimal states of function into complete loss of function, which in the extreme, obviously, is death. I couldn't be more pleased to have as expert guests this month people who really represent this whole concept beautifully and really, I believe, reflect the nature of the evolution of the functional medicine concept. These are founders and individuals associated with Cleveland HeartLab, which grew of the Cleveland Clinic, certainly—arguably—considered one of the centers of excellence in the world with regard to cardiovascular research.

Inflammatory Biomarkers are Indicators of Progressive Risk

We'll be hearing from Dr. Marc Penn and Jake Orville from the Cleveland HeartLab a little bit later in this issue of *Functional Medicine Update*. To set the tone I want to remind you that over the last decade, within the recognized etiology of cardiovascular disease it has become more and more well-respected that inflammatory biomarkers and processes are indicators of a progressive risk. Certainly we saw this in the JUPITER trial with Paul Ridker's studies at Harvard looking at individuals who had fairly low (what might be considered non-risk) LDL cholesterols, but who had elevated high-sensitivity C-reactive proteins (CRP), which is a surrogate marker for inflammation, and found that when those individuals were put on a statin that their relative risk to heart disease went down, even though their LDL cholesterols were considered already to be fairly low risk.[1] What it did is it resulted in a lowering of the hs-CRP level, so above 2 milligrams per liter hs-CRPs were associated, in the chronic state, with relative increasing risk regardless of the LDL cholesterol level. It suggests that there is this independent series of events that relates to cardiovascular risk that is associated with inflammation.

There is a very interesting recent paper that was published in the *Journal of Clinical Lipidology* titled "The Clinical Utility of Inflammatory Biomarkers and Advanced Lipoprotein Testing."[2] This is from an expert panel of lipid specialists—actually nearly 20 different experts in the field—that commented on the importance of doing relative risk analysis using an extended panel of biomarkers that incorporated inflammatory assessments. Their broadened panel included CRP; lipoprotein phospholipase A2 (or what's called Lp-PLA2, or some people call this the PLAX test)—it's really a measurement of this inflammatory biomarker that is produced by resident plaque in the arterial system; apolipoprotein B100 (apo B), which relates to the carrying of lipid associated with LDL, the atherogenic-dense LDL particle; lipoprotein a (most of us are familiar with this as another atherogenic risk factor associated with inflammation); and then lastly are the HDL and LDL subfractions (the particle number and particle count types of data that are now available from a number of laboratories to take us beyond just the gross numbers of LDL, HDL, and VLDL).

Don't Discount the Framingham Risk Factors

These are extended risk factors that really reflect more the inflammatory milieu than the traditional Framingham cardiovascular risk factors. Now, do these replace the traditional Framingham risk factors? No, the Framingham risk factors are still very viable and very valuable as gross determinants. These get down into a slightly deeper level of looking at inflammatory connections to atherogenesis and how that interrelates to the concepts of stickiness of white cells to the vascular endothelium, transluminal migration of LDL particles, LDL oxidation, foam cell formation, ultimately, then, setting up the potential for monoclonal hyperplasia of the arterial tissue and atherosclerosis. That ultimately leads to an atheroma that can have a fibrous cap and be unstable. Unstable plaque ultimately can give rise to a much higher risk to a cardiovascular accident than stable plaque. Unstable plaque relates to inflammatory mediators.

You'll hear more about this from Jake Orville and Dr. Marc Penn in the interview. I just want to set the tone: we have moved from kind of a steady-state view of atherosclerosis and its origin into this dynamic model of progression and activity that is associated with the release of inflammatory markers and the interrelationship of inflammation to atherogenesis.

I have to say that as I'm listening to myself, I'm reflecting back to nearly 150 years ago to Rudolf Virchow. He is credited in many textbooks as being the father of pathology, and he talked about atherosclerosis, which was very uncommon back in his day during the middle-to-late 19th century. In

patients he did necropsy on, he found that their atherosclerosis (what we call atherosclerosis today) looked like a wound, so he developed this wound (or injury) theory of atherosclerosis. As we get into this inflammatory sequelae in the 21st century, and now we start talking about the interrelationship of inflammation, atherogenesis, and unstable plaque, it starts looking like a wound or an injury and having some of these inflammatory mediators that are associated with injury to tissue. So, the Virchow model, revisited in the lens of the 21st century, doesn't look so preposterous. In fact, it looks like it has a very important contribution to make in our understanding, both in the late-stage understanding of disease and in its early progenitor stages as a functional disturbance at the arterial endothelial level, that one-cell-thick lining of the artery wall, where integrity of the vascular endothelium is very, very important for maintaining the integrity of what goes on inside the artery wall.

These concepts I'm describing are related a little to perspective or vision: What are we trying to ask, and what are the questions that really are on the table when you see a patient in your office? As has been often said during the training programs from the Institute for Functional Medicine, including the Applying Functional Medicine in Clinical Practice course, the lens that we view information through can determine exactly what we focus on and ultimately our conclusions. If you're really looking at pathology as your ultimate endpoint and trying to diagnose a disease, you may be looking at different markers and analyzing them in different ways than if you're looking at the trajectory towards disease, meaning the functional alterations that ultimately lead to a disturbed state of physiology in those cells that ultimately would give rise to pathology.

Does the Availability of More Tests Equal an Overuse of Technology?

What are we asking? What questions are we asking and how are we focusing the information from those questions through what lens to give rise to a specific understanding of an endpoint? That relates to this question concerning particle number and particle count in lipid assessment. There would be some individuals who might say, "Well, this is an overuse of technology. You really don't get much more definitive information by measuring particle count and particle number than you get if you just do a gross, fairly simple, cholesterol HDL, LDL, and triglyceride measurement. Why would you want to go to the difficulty of doing this more exhaustive evaluation?" It is true that within the mean of people that go on to get heart disease that lipid particle number and particle count probably is…I don't want to call it superfluous, but maybe the next step beyond that what you need for really picking at pathology. But in kind of the mid-range, where people have reasonably normal total cholesterol and they only have marginally elevated (maybe even normal) LDL levels, but die of heart disease as a consequence of having none of the traditional risk factors, should we have asked different questions? Should we have looked at their particle number/particle count to try to differentiate them from the midline of the mean? This is what some people call the "Ghost of Gauss" (Friedrich Gauss, the German statistician who talked about the midline, bell-shaped curve distribution of populations). Start saying, "What happens at the outlier side of these curves?" If you're one of those people, you'd like to know it. You'd like to know what to know what to do about it. So here's where some of this extended risk factor analysis that relates to function helps us to better understand individual personalized risk versus kind of general population risk.

There Are Different Ways of Interpreting Tests for Different Individuals

That's what many people have argued as it relates to where we're going with assessment protocols for functional intervention. Again, it depends on what we're focusing our information through—what lens and what questions we're trying to answer that really determines the kinds of things that we might be studying or evaluating in a patient. This even relates to things in the standard blood screen, like the analyte gamma-glutamyl transferase (or GGT). As you know, historically that's been considered a liver

enzyme, along with alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in a serology, and individuals have thought of this as a way of testing for drug and alcohol abuse to monitor the success of therapy. When people have elevated GGT, one follows their abstinence by looking at this enzyme in the blood to see if it is coming down into normal range.

So people have really identified GGT as kind of a toxicity from drug and alcohol surrogate measure. But there is a new interpretation on the use of GGT from Dr. David Jacobs at the University of Minnesota, who we interviewed on FMU a year ago, and his colleague, Dr. Duk-Hee Lee from Korea. What they have started to recognize, from evaluation of the Health and Nutrition Examination Survey (NHANES) data that has been compiled over the last couple of decades on American individuals and then looking at other countries as well, is that marginally elevated GGT (within the highest quintile of the normal range) is associated with increased incidence of diabetes and other cardiovascular disorders that has nothing to do directly with drug and alcohol toxicity, but really indirectly is a surrogate measure for glutathione status and oxidative stress.[3],[4] In fact, there is a very nice paper that was recently published in the journal *Atherosclerosis* which really describes the role of serum glutamyl transferase and its relationship to mortality in people undergoing coronary angiography.[5] This is a study actually done in Germany as part of the Cardiovascular Health Study. What they found in this particular study was that individuals whose serum levels in the upper quintile of GGT in the normal range had a significantly increased predictive all cause in cardiovascular mortality versus those who had the lower normal levels of GGT. And they again tie this to other risk factor markers other than alcohol and drug toxicity that are associated with cardiovascular disease origin, or the etiology of atherosclerosis.

What would this be? Well, that takes us into a functional model rather than a pathological model in which we say the GGT may be a surrogate marker for oxidative injury, for mitochondrial uncoupling, for oxidative stress , and on toxicity and therefore we might start looking in a different place to answer the question as to atherosclerosis in that patient other than the traditional cholesterol risk factors. HDL is a functional protein that is made up of apolipoprotein A-1 (apo A-1) and 40-plus other different proteins that give the HDL particle a personality that's very different than the LDL or the VLDL particles, or the IDL particles. The HDL is a functional protein: it has antioxidant capability, it has anti-inflammatory capability, depending upon the personality of the various proteins that are found within the HDL particle. The differentiation of composition of the HDL is more than just the number, so we can't really say the HDL number, let's say 50 or 45 milligrams per deciliter, isn't the whole story. In fact we know there are people in Italy--in the Limone Sul Garda region--who have very, very low levels of HDL, but yet have very low incidence of cardiovascular disease, and it is found out that their HDL, even though it is low, is functionally very capable based on the protein composition of their HDL particle of engaging in cholesterol efflux and pulling cholesterol out the artery wall and lowering serum cholesterol effectively. It's a measurement of HDL function as well as HDL number that becomes very important for determining relative risk.

We're going to hear much more about functional tests for HDL. This was a big discussion by Jay Heineckein the *New England Journal of Medicine* recently in the January 2011 issue, in an article he authored that discussed the role of functional HDL in cardiovascular disease protection versus just looking at the HDL number.[6] I think this is a wonderful segue into what we are going to hear from the experts from Cleveland HeartLab, Jake Orville and Marc Penn, in which we'll be discussing how we use biomarkers in the laboratory to more effectively evaluate relative risk as it is associated with dysfunction of the cardiovascular system, as contrasted to just looking for cardiovascular pathology. So we moved the sequence of our insight back maybe decades to an earlier time where intervention can occur much more mildly and we can follow much better the trajectory in that patient's health to avert a later stage necessity for intervention with stenting or surgery. With that, let's move to our discussion with our

clinician/researchers of the month.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month

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Here we are once again at that point I look forward to with great anticipation and that's our Clinician or Researcher of the Month. I'm very fortunate this month. Actually in my office sitting with me are two luminaries that are really doing things that I would say are on the cornerstone of what we've been talking about in functional medicine for the better part now of 30 years: Jake Orville, who is the President and CEO of Cleveland HeartLab, and Dr. Marc Penn, who is an MD/PhD in cardiology and also an expert on the whole field of stem cell research as well. So, some very interesting basic science as well as clinical background.

We've been talking today about what's going on in the theranostics area, the early stage assessment area, how one looks at function prior to the onset of pathology. What are the rising tides in this field that can be more capable of uncovering occult trajectory towards disease to both lower the expense of disease management and of course, humanistically, to improve quality of life and quality of care? Both Jake and Marc, thanks a million for being with me here this afternoon. Let me throw out the first question. What took you two down this road with Cleveland Hear Lab to start moving away from maybe the more traditional cardiovascular Framingham risk assessment markers into some of these more esoteric or functional-based markers?

Establishment of Cleveland HeartLab

JO: Sure. Jeff, thank you for having us. I think it all started with our idea that we could do better, and the traditional methods—at least we were being told by physicians—maybe weren't good enough. So we got together, Marc and I, and really thought about innovative novel testing that is additive to the traditional methods of testing to see if we could really help practitioners identify those that were really at risk for disease.

JB: Marc, you have a very interesting joint background: basic science as well as clinical work. How did it pull your interest into the field?

MP: I think what I recognize is that we have made tremendous strides in treating lipids in patients and decreasing the risk for heart disease, but now that about 50% of the patients who present with heart attacks have normal lipids, either treated to or naturally, I think it became clear that we needed to look under a different rock, if you will, in order to define who still has residual risk even though you've

treated them to what the guidelines state for lipid management.

A Brief Chronology of Establishing Cardiovascular Risk Factors

JB: I'd like to go back with the two of you and do a little bit of a quick chronology. I want to go back first to Framingham, and then from Framingham I'd like to go to Brown and Goldstein, and then from Brown and Goldstein I'd like to go to Paul Ridker, just to kind of get a connector of beads on a string that makes a necklace. If we go back to Framingham and look at the work that was done in Massachusetts, it set the tone for risk factors and how that would be woven eventually into medical management, and then how that tied with blood cholesterol. Boehringer, I think, was the first company to develop a finger-stick cholesterol test that made it accessible at health fairs and suddenly the cholesterol number became a person's number because they could get it at their shopping mall. That technology, then, ultimately drove probably the largest singular drug family in the pharmacopeia today, the statin drugs. From your perspective as a cardiologist, Marc, give us a little bit of your lens as to how you have seen the evolution of this field.

MP: Yes, and I think you're exactly right. Framingham set the tone for risk factors, recognizing that certain patients will be at a higher risk of developing disease. The other milestone I would put in your list is Russell Ross's response-to-injury hypothesis in '76, which really set the tone for studying lipids and how they induce injury, and then studying inflammation and how they propagate the disease. Brown and Goldstein, in their seminal work, recognized the lipid portion as inducing that injury, and perhaps driving the propagation. And then Ridker coming back and recognizing the inflammatory part of the pedestal, and recognizing that even in patients whose lipids are okay, if they have arterial inflammation they are still at risk. I think you've laid it out very nicely and I think that really is an amazing history in the era of medicine that had tremendous effects on changing the disease process.

JB: Why do you think that there has been such a push back in medicine? I heard Paul Ridker speak not too long ago and his work seems quite impeccable on the inflammatory connection to atherogenesis, yet there are some very vehement members of the cardiology community that are totally resisting these extended factors. Why do you think that is?

Resistance to Acceptance of Extended Risk Factors

MP: I'm not certain why. Cardiologists are a challenging group to change our mind. I'm a cardiologist and I feel that way, I guess, too. I often wonder if the reason CRP is not well accepted is because we were all trained on the classic CRP, and these values are all within the normal range of the tests we were all taught. So there is always a resistance to changing something we learned, and if it were a new test called something else maybe people would be more accepting of it. But I agree, I think Paul's work is impeccable. I think it's revolutionary and he's really moved the field forward in many important ways. I personally use it and I think it is an important strategy in anybody's armamentarium who is truly going to try and push prevention, either primary or secondary.

JB: I often hear some docs say, "Well, you know I tried measuring CRP in my patients, but it seemed to jump all around because maybe they had a cold, or maybe they went out for a long run." As a clinician, how do you respond to those comments?

MP: I can only go by my own personal experience and I think the test behaves fairly well. If somebody comes in sniffling and sneezing it is not the time to measure CRP. I think, you know, one of the debates I've heard is, "Well, my patients have rheumatoid arthritis and their CRP is high." Well, if a patient has rheumatoid arthritis they have a five-fold increase of MI anyway, so we should be treating aggressively and that CRP is, in fact, reflecting that. Part of it is an education piece, part of it is an open-minded piece, but again I think the data are pretty strong. Personally I think I've had good success with it.

JB: So now let's go to the JUPITER trial. What's wonderful about the field that we share is it never

suffers for lack of controversy and back and forth. People take the same study and they get polar opposite, diametrically different opinions about it. Tell us—from the view of an expert—about the JUPITER trial.

MP: What I find interesting about the JUPITER trial is that really the breakdown of those who've got a CRP under 2 and those with an LDL under 70. Obviously if both an LDL was high and a CRP was high in patients, they didn't have any benefit compared with controls. If either the CRP or the LDL were below that cut-off they did better. What I find really interesting is if the LDL and the CRP were lower, they did yet better, which really does suggest that if one chooses to just measure lipids, they are choosing to miss half the story. And while certainly I think in a lot of people's minds the lipids drive the inflammation and if you know where your lipids are you can figure out where your inflammation is or vice versa, but reality is the data repeatedly, in multiple studies, would suggest knowing where your patient achieves a lipid status and what their inflammatory status is are actually additive information, they are not redundant, and it truly helps us fully describe the risk of that patient in front of you.

Biology versus Anatomy of Vascular Disease

JB: So I've heard you eloquently talk about the biology and the anatomy of vascular disease and how one can see it progressing both anatomically and also physiologically, and different markers provide different types of insight into that process. Could you talk a little bit about this lipid versus inflammation connection in that context?

MP: Sure, so you know there are repeated studies that have either looked in the coronary tree using calcium scoring and then looking at plaque activity with myeloperoxidase, or with the Lp-PLA2 test (the PLAC test), showing that if you have good anatomy but you have bad biology (a high myeloperoxidase) you have risk. If you have bad anatomy and bad biology you have even more risk. So clearly, again, this concept of if you choose to pick one or the other, cardiologists are very good at the anatomy side with cardiac catheterization and computed tomography angiogram (CTA), and you're limiting, really, fully describing the risk of that given patient.

JB: I'm going to put my little spin on this and you can correct me if you feel I've over interpreted, but I would put the spin from the functional medicine perspective that the biology refers to function and the anatomy refers to pathology, right?

MP: Yes, I think that's very fair.

JB: Okay. So what we would say in the functional medicine model is that dysfunction precedes pathology.

MP: Yes, and certainly if you look at our offering of F2-isoprostane as a general marker of oxidation, that precedes pathology, but if we have that abnormal function, the studies will tell us that the pathology will follow.

F2-Isoprostane as an Analyte

JB: So let's talk about the F2-isoprostane. I think that's a really interesting analyte. Early on, as I recall, when that was first in the literature, people were talking about it as an oxidative stress marker—as a kind of an interesting prostanoid-like molecule that came from reactive oxygen hitting an eicosanoid structure to form this. Do we interpret it differently today as we've learned more about it?

MP: No, I think we recognize it possibly as the gold standard measure of oxidative stress and how much oxidation is going on. When we look at deconditioned patients and recognize that they have high F2-isoprostanes, and we recognize that as they begin to exercise that actually goes up because they're burning more fuel, but as they become physically fit, it goes down because they are burning less fuel per

unit of work. We recognize that it really does reflect the functional physiology that's going on, and if we allow it to maintain a high state in a deconditioned patient, we're working our way towards the pathology.

JB: Let's now then go back and review because we're going pretty quickly, probably, for the listener who is trying to keep track of this. We have the F2-isoprostane. We have the CRP. We've got the myeloperoxidase and we've got the Lp-PLA2 (the PLAC-type analysis). How do they fit together, then, in a schematic representation?

MP: What we would suggest is that F2-isoprostane is really defining the patient who has a lifestyle risk: they are deconditioned, they smoke, they have a bad diet. That leads to increased oxidative state, increased risk long-term of not only cardiovascular disease but also cancer. If we're now looking for presence of marker of disease, we're really looking at CRP, which based on our studies would really be a measure of atheroma burden, and then albumin/creatinine ratio, which is really a measure of endothelial dysfunction. Working our way from lifestyle to presence of disease to activity of disease, now we're looking at the myeloperoxidase (MPO) and the Lp-PLA2 (or the PLAC test), which are really looking at vulnerable plaque formation from really two different points of view. The Lp-PLA2 (or the PLAC test) really looking at it from the vessel wall point of view or "inside the house," if you will—looking at the activity in the necrotic core, macrophage activation, things of that nature. And the myeloperoxidase test is really looking at it from the lumen side—what the white cells are saying—"outside the house," if you will: fissures, erosions, hot atheroma that is starting to come through a collagen cap. Together, this really not only helps us to find whether a given patient has risk, but where they are on a risk spectrum: Are we really focusing on lifestyle? Are we trying to modulate disease? Or are we really trying to quiesce the function that is going on in the vessel wall that is risking a clinical event?

JB: I have often heard docs worry about "over utilization" or "over testing." They'll say, "Gee, I wonder if that's a standard of care? I wonder if I'm doing too much testing?" It sounds like when we have a portfolio of tests it gives us different ways to look into the lens of the progression of cardiovascular disease. What would you say to a doctor who says, "Am I using too many tests?"

MP: The reason we focused on a panel approach at Cleveland HeartLab is the studies have demonstrated that these tests offer additive information. There is an elegant study by Heslop and colleagues out of Canada last year that showed that if you had a high MPO level your ten-year risk of mortality was significantly increased.[7] But what they also ended up showing was that if you had a low MPO and a low CRP, you did well. If either were high, you didn't do so well. If both were high you did yet worse. So it is hard to say that defining a MPO and a CRP are redundant or over testing. Similarly, we've looked at data in over 2000 patients from executive health programs and preventive cardiology clinics where about five-and-a-half percent of the patients will be at risk based on high MPO, and about four to four-and-a-half percent will at risk based on a high Lp-PLA2, but yet despite having well over 2000 patients, only six patients had both markers up. So it is hard to argue that those tests are redundant when you have such high discriminatory values. By random chance you would think you'd have more than six patients up in that kind of population, yet we did it. What we say—and it is consistent with the biology and consistent with our understanding of the pathology and the function—is that if you have a hot vessel wall, and you have white cells responding to that, you're not in an executive health physical. You probably have acute coronary syndrome if it is in the coronaries. So I don't see—and we've developed this panel very specifically—not to have redundant testing, not to be over-testing, but really to, in a very logical and rational way, taking pathology and function into consideration, a panel of tests that allows us to define where our patient is.

JB: That was a brilliant explanation—very succinct—but you also used a term, there, which I think is a very interesting term, and that was in the context of a "syndrome" (acute coronary syndrome). Why don't we

call that acute coronary disease? What's the difference, there, in the languaging of that as a syndrome?
MP: I think the reason it is a syndrome is it can be caused by multiple different effectors. You can have a hot vessel wall that ruptures. You can have fissures and erosions that allow platelet activation. You can have a flu-like syndrome that causes active plaque. You know, it is just not a single event. It ends at the same place: you have plaque rupture, the growth of lesions, and/or thrombosis. But there are multiple avenues to get into that state.
JB: I've heard Dr. Mark Houston say something like, "There are an infinite number of causes with a discrete number of biological responses." Does that make sense that the body has a discrete number of biological responses to a whole set of offending precipitators or initiators?
MP: Yes, I hadn't heard that, but I think that's excellent, actually. We clot, we attack infection, we don't do that many different things.

JB: So Jake, from the perspective of getting to docs and helping them understand this emerging new biology of vascular disease and how these analytes can be useful in helping them be more specific in managing patients, what are your challenges and how do you overcome these in getting people to understand, people who may be trained in a different perspective and this is all new to them.

JO: The biggest challenge is education, and education starts with: What are these tests and what do they mean? Then it goes to: What are the results and how to interpret? And beyond that, which is really a focus of our company: What do you do next, and what do you do with the patient, and when should you see them? So after that patient leaves the office, that practitioner has a good understanding of what they have just gone through, an explanation to the patient of what they should do, and then a clear expectation of what they should do after they leave, and when they should come back. I think that's one of the biggest hurdles. You don't necessarily take that educational piece and put it on your back as a service laboratory, but we really believe strongly that that is where we should focus the most.
JB: It seems interesting to me, as I just—from one step removed—watch what's going on in the specialty lab area in cardiology, you've got Berkeley Heart Labs recently (not real recently, but reasonably recently) acquired by Celera, which sounds like a very interesting jewel to put on their crown, and you see others, like Atherotech and LipoScience being acquired as investments from companies that you normally might not think would be focused on functional cardiovascular diagnostics. What does this say? What does it say about the field? What does it say about the trend?
JO: To me, I think people are trying to go as far upstream as possible to get the information, to understand what's happening, so they can design therapeutics, design supplementation—nutraceuticals, foods, what have you—that attack what is happening in the body and I think it all starts with a test. If it was as easy as us just looking around and saying "You're at risk and you're not" we wouldn't need this, but we need a lot more information than that. I think it has been a great effort by a lot of the labs you mentioned, which are the great groups that are out there really doing innovative things, and I think we're all working together to show that getting a diagnosis or getting a prognosis will help the downstream efforts of not just the physician, but the companies that are then going to help support the physician afterwards.
JB: And I think part of it drives what you said earlier about patient education: tools that the patient can use to kind of understand their body at a different level so they become more invested. If it is somebody else's body you just drop it off in the exam room and walk away, you don't maybe have the same investment as to owning and understanding what's going on.

JO: That's right.
How Important are Particle Number and Particle Count?

JB: Marc, one of the things that strikes me as interesting about the evolution of this field is you've got debate going on around lipid particle number and particle count and whether this is really going to be helpful in personalizing therapy or it is just another window dressing. What's your perspective on that?
MP: Well, I think the concept of particle number is interesting and important in, clearly, a subset of patients whose particle numbers do not track with the classic lipid panel. Their apo Bs are high. You really do identify an insight into that patient that you wouldn't otherwise get. The same is likely true for HDL and HDL particle numbers. So, again, I think in this era of specialty testing, if you identify patients who have a risk, I think you can better describe what that risk is and more importantly what your therapeutic response should be based on those kinds of tests.
JB: Tell me a little bit about this interesting particle, the HDL. When we learned about apolipoproteins and lipoproteins, I think we all had this thought that maybe they only differed in density and size. But now we have learned that this HDL is this very complex particle with 40 or so proteins that make it up. Tell me a little bit about how the HDL differs from the LDL, the VLDL, and the IDL.
MP: I think what's interesting about HDL or what is becoming clearer is it's not about how much HDL you have, it's how functional your HDL is, right? Folks with apo A1-milano have relatively low HDL levels, but what is clear is their HDL is relatively oxidant resistant. It stays functional longer. It fluxes through the body better, so it can pick up lipids from the vessel wall and return them to the liver through LDL in a more efficient manner. I think we learned a tremendous amount through the Torcetrapib experience with the cholesterol ester transfer protein (CTEP) inhibition and the fact that, you know, if you do a mass balance on CTEP inhibition you might argue that you're not sure it's going to work, but I don't think anyone expected it would be hazardous. And clearly an accumulation of dysfunctional HDL particles appears to be proinflammatory, and certainly (in that study) seemed to have negative consequences. Given the degree of blood pressure changes, it doesn't seem to many, including myself, that the adrenal effects were really where the negative effects were. Now it may be that other CTEP inhibitors that are not irreversible, that are partial inhibitors, maybe they will be successful. I know they are certainly going forward in clinical trials and it will be exciting to see. And then what we have learned is that even in the absence of CTEP inhibition, apo A1 can become oxidized. There is some very beautiful work by Jay Heineke that has demonstrated that oxidized apoA1 cannot participate in reverse cholesterol transport.[8] So we are learning that these folks who have inflammation may have low HDLs, but it may be functionally much lower than we think it is because in fact a lot of the HDL they have may not be functional. I think we're learning. We're working diligently on dysfunctional HDL assays. And I think as physicians we're going to finally learn not only what percent of our patients have dysfunctional HDL, but which one of our therapies improves that. Because right now it's a black box: Is it fibric acid derivatives? Is it stains? Is it niacin? We just don't know, and I think it's going to be a very exciting time when we have a dysfunctional assay out to not only define who's really at risk, but also who responds and what the right therapeutic target should be.
JB: That was really very, very insightful. For the listener—just to kind of fill in a little bit of the lexicon—CTEP transfers the cholesterol out of a lipoprotein to another site. The Torcetrapib trial had Pfizer ready to roll out to market this new drug that was going to extend the patentability of the atorvastatin via combo HDL-elevating molecule coupled with LDL cholesterol lowering molecule. That 800 million dollar risk adventure failed, for the reasons Marc was just describing. That begs a question on this whole news about niacin, because niacin was also one of these pharmacological agents (the therapeutic dose) that was an HDL elevator. Do we see the same thing going on there as with Torcetrapib?
MP: Well, Torcetrapib raised HDLs for sure, but where CTEP inhibition came into play is that HDL can pick up the cholesterol from the artery wall. It ultimately transfers that cholesterol from the HDL to LDL

in the bloodstream. The LDL actually completes the circuit by returning the cholesterol to the liver. There was hope that if you block cholesterol ester transport protein, you raised HDL levels and in fact you raised HDL levels very well. The question was: Could the scavenger receptor B1 (SR-B1) in the liver take up all that HDL? Would it go there, and would it be efficacious? Even on the patients with Torcetrapib who raised their HDLs 60, 70, 80 percent, they actually had an increase in mortality because there was a lot of this HDL around that could not return their cholesterol. As near as we can understand, that HDL became dysfunctional, and actually was then probably proinflammatory and caused vascular events. With niacin we're not seeing that. Niacin is driving more HDL to be made. It is not inhibiting the flux; it is hopefully enhancing the flux. The vast majority of trials with niacin have been positive. Recent carotid intimal medial thickness (CIMT) trials were not positive. There is a debate as to whether they were the right patient populations which truly had risk. And they were also well-treated going into the trial, so there wasn't a lot of atheroma to try and reduce. But niacin is certainly safe, and seems to be efficacious in the majority of trials.

JB: One last question which I think bears on this mosaic of confusion, and that is statins. We have this concept of a class effect. The more one gets into pharmacology, the more a person starts saying, "Is there really such a thing as a class effect? Is that just kind of a marketing language?" Because each of these molecules that are within a class, if they have a different structure they have a different function. So we think, is Crestor the same as Lipitor (or whatever—choose your statin of the day)? Could you tell us a little bit about how you see things evolving as it relates to the difference among the class of statins.

What Do Statin Trials Tell Us About Class Effect?

MP: I think if you take the field as a whole and you look at outcomes, you have trials like the ASCOT trial that looked at 10 milligrams of atorvastatin and you compare that to the Scandinavian Simvastatin Survival (4S trial) which looked at twenty milligrams of simvastatin. Simvastatin 4S reached a mortality end point; ASCOT did not. They had about the same level of LDL lowering, so you start to wonder why. Well, simvastatin tends to be a better HDL-raising statin than atorvastatin, so maybe it's that. You then look at ASTEROID, which was 40 milligrams of rosuvastatin, which was an intravascular ultrasound study, and you compare that to the REVERSAL trial, which was 80 milligrams of atorvastatin. Well, 40 milligrams of rosuvastatin has roughly the same LDL-lowering effect as 80 milligrams of atorvastatin, and you say, "Well, why did ASTEROID see regression and REVERSAL didn't see any regression?" Well, rosuvastatin is a pretty good HDL-raising statin and atorvastatin is not a particularly good HDL raising statin, particularly at 80 milligrams. So you start seeing themes around the biology (the picture, the anatomy) and then the function, or the pathophysiology, between ASCOT and 4S. Steve Nicholls, who was a colleague of mine when I was at the clinic, published a very nice study several years ago, now, that looked at intravascular ultrasound studies and what changes in lipid parameters predicted regression.[9] What Steve found was that if you got the LDL under 87.5 milligrams per deciliter, there was evidence of regression. But what he also showed, which was very interesting, was if you raised HDL, it's 7-and-a-half percent, so that's 40 from 43. It's not a big change. There was also significant benefit. So again, if you take 4S and ASCOT, REVERSAL and ASTEROID, and you say, "Well, maybe the HDL effect is real," and then you say, "Well, how do you get 7-and-a-half percent rise in HDL?" Almost any dose of simvastatin will get you above 7-and-a-half percent. Any dose of Vytorin will get you above 7-and-a-half percent. Five milligrams and above of rosuvastatin will get you 7-and-a-half percent. Almost no dose of atorvastatin will get you 7-and-a-half-rise in HDL. It becomes a very interesting concept of: Is it the HDL effect of the statin and therefore, then, it is not a class effect? And, again, when we have a dysfunctional HDL assay and we can say, "Not only did we raise HDL, but actually 20 percent more of it was good," we'll be able to make better conclusions along these lines. Because it does appear that the

HDL effect of a statin, as modest as it really is, seems to predict the biologic and functional response.
JB: That's really insightful—very, very interesting. I guess it is a theme that would play, also, with other interventions—lifestyle interventions or anything that is going to influence your lipid number, particle size, and distribution, you could use that same logic, it would seem.
I said that was the last question, but I actually have one more follow on. We've always thought of this as the "Lipid Hypothesis." But I recall an article that was written back in the mid-to-late 70s called the "Lipoprotein Cascade."[10] I think it was by Eaton. He was proposing that atherogenesis is also an apolipoprotein issue as well as a lipid issue, and that there are many things that influence the biosynthesis of apolipoproteins: it could be stress, it could be hormones, it could be insulin (a hormone, I guess, but I was thinking of steroid hormones versus peptide hormones). And so we ought to be looking at it from the apolipoprotein perspective as well as the lipid, because the lipid is carried, obviously, by apolipoprotein. Do you have any comment about that kind of duality—lipid versus apolipoprotein?
MP: Yes. Part of it is the dysfunctional part of HDL and apo B certainly is all about the apoprotein (the oxidation). It's the protein; it certainly is the part. We also have some interesting data that will be coming out soon that apo A-1 may play a significant role in the uptake of Coenzyme Q10 (CoQ10). We've now looked at mice that cannot make apoA-1, and in a gene-dose dependence, it actually regulates how much CoQ10 is actually taken up in the heart. And if you actually induce a heart attack in these mice, their infarcts are huge—much greater than normal mice. But if you supplement them with CoQ10, you actually normalize their infarct size, and we've now mapped this to a very specific deficiency in the mitochondria. It's quite clear to us now that apo A-1, the apoprotein, does have quite a significant role in CoQ10 absorption. If that turns out to be clinically valid, which I think there is evidence it is, you almost have to reinterpret Framingham to recognize low HDL may not just cause more heart attacks, but it may actually cause bigger heart attacks, and we may be raising HDLs for all of the reasons we don't understand, which is to actually improve the absorption of CoQ10, replete the mitochondria in the body so that if they have an infarct, their infarct is smaller.
JB: That's fascinating. So Jake, I'm going to give you the last word. You've got the responsibility as president/CEO of Cleveland HeartLab of kind of directing the future implementation of this wonderful new science that is becoming available. What do you see on the landscape, in front of you and your group?
JO: I think you two have discussed where we want to focus, which is the function. I think we see a lot of novel markers that predict risk and I think there are a lot of those that are out there. I think we're trying to decide: Does it have a good risk? Can it give insight on what to do? And what is the function of it? That's where we are at. Clearly there has been a strong interest in the educational side of things and complement the biomarkers, and if we can continue to focus our areas on functionality and the additive nature of risk and what happens next I think we'll do very well. I think we will continue to be adopted and embraced out there in the physician community.
JB: Are you seeing much push back or receptivity to these panels being included within various insurance reimbursement diagnostic code programs?
JO: Just to clarify the definition of a panel: When Marc suggests a panel, that's for educational purposes. We don't sell panels. We provide testing and we provide education. It's really up to the physician to kind of form their own panel. Marc provides a lot of education on panels that may be insightful. From an insurance company standpoint, obviously we want to make sure that they are aware of this testing, that they are aware of the value and the benefit, and I think it's a question you asked several questions back, which is: Is too much testing a problem? I think our answer is "yes," which is why we go right to the root, which is what we believe to be inflammation, and then follow the risk backwards. Let's find out where this patient is—if they are at risk—and I think we have seen a good embracement of that from the

insurance companies, to understand what it is to go right to the heart of the risk, first in an affordable manner and then decide where to go from there.
JB: Very well said. I want to thank you both. I think this has been really a lot of news-to-use in a very concise fashion. Thanks a million, and we're going to be checking back. This is actually the pulse point of where functional medicine and functional cardiology is going. Thank you both.
JO and MP: Thank you

Bibliography

[1] Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359(21):2195-2207.

[2] Davidson MH, Ballantyne CM, Jacobson TA, et al. Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. *J Clin Lipidol*. 2011;5:338-367.

[3] Lee DH, Steffes MW, Jacobs DR Jr. Can persistent organic pollutants explain the association between serum gamma-glutamyltransferase and type 2 diabetes? *Diabetologia*. 2008;51(3):402-407.

[4] Ha MH, Lee DH, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: results from the National Health and Nutrition Examination Survey, 1999-2002. *Environ Health Perspect*. 2007;115(8):1204-1209.

[5] Stojakovic T, Scharnagl H, Trauner M, et al. Serum gamma-glutamyl transferase and mortality in persons undergoing coronary angiography—the Ludwigshafen Risk and Cardiovascular Health Study. *Atherosclerosis*. 2010;208(2):564-571.

[6] Heinecke J. HDL and cardiovascular-disease risk—time for a new approach? *N Engl J Med*. 364;2:170-171.

[7] Heslop CL, Frohlich JJ, Hill JS. Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography. *J Am Coll Cardiol*. 2010;55(11):1102-1109.

[8] Shao B, Cavigiolio G, Brot N, Oda MN, Heinecke JW. Methionine oxidation impairs reverse cholesterol transport by apolipoprotein A-I. *Proc Natl Acad Sci U S A*. 2008;105(34):12224-12229.

[9] Nicholls SJ, Tuzcu EM, Sipahi I, et al. Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *JAMA*. 2007;297(5):499-508.

[10] Eaton RP. The lipoprotein cascade: clinical implications of hyperlipemia. *Continuing education for the family physician*. 1977:42-49.p>