Bob Barrett: This is the podcast from Clinical Chemistry. I'm Bob Barrett. Human C-Reactive Protein or CRP is a liver synthesized blood constituent that increases rapidly and robustly in cases of tissue damage, infection and trauma.

For years, increases in serum concentrations of CRP have been used to gauge inflammation. With the introduction of higher sensitivity laboratory assays, it's become apparent that even a modestly increased serum CRP in healthy individuals correlates with risk of cardiovascular disease. In most cases this relationship is independent of traditional risk factors including age, smoking, high blood pressure and high cholesterol concentrations.

Despite the evidence that CRP is associated with inflammation and the risk of cardiovascular disease, it is not certain how it does so. A recent paper by Fujita and colleagues published in the October issue of Clinical Chemistry provides a new clue to help answer these nagging questions. Dr. Alex Szalai, a professor of medicine and microbiology at the University of Alabama at Birmingham broke down these new clues in an editorial that accompanied that publication. Dr. Szalai is our guest in this podcast.

Doctor, CRP is known as an acute phase protein. Can you explain what that is?

Alexander J. Szalai: Acute phase proteins in general are really just proteins that -- usually most of them are made by your liver, and they're dumped into your circulations.

So these are blood proteins that are made in the liver. And if these proteins changed the concentration in the blood by several fold in response to an injury or an infection that causes inflammation then they're known as acute phase proteins. So C-Reactive Protein is actually the first acute phase protein described, so it's the prototype of the whole class of proteins.

And just to give you an idea at baselines in healthy humans with no infection or no injury, it circulates at a level that's about one microgram per ml. If you do have some sort of injury or some sort of inflammation, it it's severe enough, those levels can increase up to a 1000 fold. And it can increase in a matter of a day, 24 to 48 hours.

So CRP is not only the prototypic acute phase protein, it is the benchmark acute phase protein. It's the one that has the most robust, most rapid change in blood level.

Bob Barrett: A number of studies have demonstrated that CRP is associated to cardiovascular disease but this linkage is not as an acute phase protein, why is that?

Alexander J. Szalai: First of all the interesting thing because CRP since its discovery which was really back in the 30s -- in fact, the reason it was discovered is because its levels were high enough during infection in this case it was what was called diplococcus bacteria, the streptococcus that causes pneumonia. Its levels are high enough that you could detect it with rather crude assays. So the expectation would be that it's the high levels during inflammation that would associate with diseases and that is the case, as I told you, it's associated with inflammation.

The surprise was, when technology advanced and we had the tools, the software and hardware, where we could pick a blood sample and measure many, many samples of blood in healthy people and ask a simple question how much CRP do we make when we're healthy? The surprise was -- one of the unexpected outcome was that measuring CRP at baseline and as I told you in North American Caucasians at somewhere in the 1 to 3 microgram per ml range typically. But right now if the listeners are healthy – the amount of CRP we make when we're healthy that is a very strong predicator of our heart health 10 years down the road.

So it's baseline CRP that is associated statistically with outcome. The outcome being cardiovascular disease, so that was the surprise. So despite the fact there's a protein that can go from low level to very high levels rapidly in the face of overt inflammation, it's the levels that we make day-to-day when we're extensively healthy that predicts best who will, who will not have heart diseases right now.

Bob Barrett: Would the CRP cause heart disease and if so through what mechanism?

Alexander J. Szalai: That's actually the – I'm not sure a million dollars is enough nowadays to speak, it's the several dollar question. If it does, the association indicates smoke, for example, from a fire.

That's an association or is CRP the fire, is it part of the problem. And people have been working for many decades trying to really nail that down. In humans I could tell you there's really no direct evidence in humans that CRP causes heart disease and the reason for that is because we can't do the proper study. There's no drugs available yet where you could do a placebo controlled study and for example take people with CRP levels and specifically target CRPs, so that you can lower CRP and look at outcomes.

So what people have done is look at animal models. In animal models the evidence is increasingly strengthening. And at least in animal models CRP does contribute cardiovascular disease. And what CRP is doing in that context is based on its structure and that's the unique thing about CRPs. CRP is a pentameric protein so it seems like cherry blossom, like a flower with five petals. And on one side of that the protein combine to ligands expressed by bacteria such as – the best one known is phosphocholine, that same ligand can be expressed by things like lipoproteins, LDL, HDL, which most people understand the levels of those lipoproteins can predict heart disease. The ligand can also be expressed by damaged cells. So you can see how CRP can interact with many components that are known to be involved in heart disease and all of those interactions on the side of the flower that I used as a metaphor. That's called the B face or the binding face.

The other face of the protein or the other side of that flower can bind to receptors on the cells called Fc receptors. So you can see how the protein can bind a lipid for example and then deliver it to a cell. And that same side can also activate system of proteins in your body called complement in particular the complement protein called c1q. So the protein is pentameric, one side of it binds to things. The other side has the activity and it's known as A face for the activity face.

So a heart disease, now to – and actually heart disease I mean things like stroke, and angina, myocardial infraction, atherosclerosis, they're all increasing becoming known as diseases of inflammation. And inflammation as I said drives the acute phase response, so there's a good logical reason to expect that CRP might contribute to cardiovascular disease.

But having said that, again I just want to reiterate that until we have a specific drug and there are some in the works, a specific drug that you can give to somebody to specifically lower CRP, we won't really in humans if CRP contributes to heart disease. The animal studies on the other hand are pretty convincing that it does.

Bob Barrett: Doctor what is LOX-1?

Alexander J. Szalai: LOX-1 is another receptor. It's an oxidized LDL receptor. LDL is Low Density Lipoprotein. It's the nasty lipoprotein. So we have a receptor called LOX-1 that is expressed on several cells that was discovered on cells that lined the vasculature endothelial cells. So endothelial cells that lie in arteries, in your veins, they express LOX-1.

LOX-1 interestingly enough is already known or for several years has been known to be associated with heart disease as well. And it can do a lot of the things I just described for CRP. LOX-1 as the name implies can bind LDL, in particular it can bind Low Density Lipoprotein that has been oxidized and CRP can do the same thing. LOX-1 can also bind damaged cell, CRP can do that, LOX-1 can also bind bacteria and CRP can do that. So LOX-1 independently, independent of CRP that is, has been something that people have been investigating as a known marker of cardiovascular disease. And again people have considered the possibility that may contribute to cardiovascular disease.

Bob Barrett: They seem to have similar characteristics but is there a working relationship between LOX-1 and CRP?

Alexander J. Szalai: That's really the article by Fujita and his colleagues, actually a series of articles but the most recent data looking at that question and what that did is they established first that C-Reactive Protein does indeed bind to LOX-1. So CRP can bind to that oxidized LDL receptor and it can not only bind but it can actually with tests using cells in vitro in a test tube or with animal models again that that interaction between CRP and LOX-1 actually is meaningful. Meaning it can change the function of the endothelial cell where LOX-1 is expressed in a way that one would predict would contribute to cardiovascular disease.

So here we have two proteins C-Reactive Proteins which circulates in the blood, LOX-1 which is expressed on cells that line the blood vessels and historically both of those have been known to associate with heart disease. And their paper and their initial studies showed that these two proteins interact, they possibly could be telling us the same thing, that the CRP effect, CRP driving cardiovascular disease may in fact be through its ability to bind LOX-1.

The other important thing is that remember LOX-1 binds oxidized LDL and CRP binds oxidized LDL and there is good evidence that oxidized LDL is the culprit that drives things like atherosclerosis for example. So you've got CRP and oxidized LDL using the same receptor LOX-1.

Bob Barrett: So doctor regarding the paper in Clinical Chemistry by Fujita and associates, what do you see as those important findings?

Alexander J. Szalai: Well in this paper in Clinical Chemistry, the authors ask the next really important question, questions actually there were two. One is, is LOX-1 promotes the CRP effect that I alluded to. If LOX-1 really was necessary for CRP's influence in cardiovascular disease, the first question was how does

CRP interact with LOX-1? Showing that the two interact is not the same as showing how they interact.

And the second question was, does CRP interacting with LOX-1 turn on one of those two known activating pathways that I mentioned earlier, the Fc receptors or the complement activation pathway. And in this paper in particular, they asked the question does CRP binding LOX-1 activate the complement system?

And what they showed in this paper that really was novel was that CRP does, as I said earlier and as they showed in their earlier papers, CRP binds LOX-1. The surprising thing was that when CRP bound LOX-1, it activated one of the complement protein C1q. The second surprising thing or unexpected thing, perhaps we shouldn't be surprised by nature, the unexpected thing was the way that LOX-1 and CRP interacted was novel. And what I mean by that is the face of CRP, remember the petal shaped pentamer, the face of it that interacts with phosphocholine damaged cells, with oxidized LDL was in fact the same face that interacted with a LOX-1 receptor.

That's novel because that's the only receptor so far that is known to interact with CRP in that fashion. The other side of the CRP protein is the side that interacts with Fc receptors or C1q but not both. So that brings up another twist to this old protein is -- again if you imagine the cherry blossom, if that CRP and then complement is a bee and Fc receptor is a humming bird, you can have the humming bird or the bee at the flower but you can't have both and CRP works in the same fashion. CRP will either engage complement and activate it or it will engage Fc receptors or activate it.

And both of those actions are downstream actions of the other face having interacted with a bacterium or with an apoptotic cell or a damaged cell. So it's kind of – you can see there is a three step interaction, you've got a target, CRP binds to it and then that CRP that's bound can engage complement or it can engage Fc receptor.

What the LOX-1 story adds to this is a unique three step pathway that goes in the other direction. So you've got LOX-1 which is an endothelium receptor but now LOX-1 or a phosphocholine or a damaged cell can bind and compete perhaps like the bird and bee analogy but compete for binding to other face of CRP. So the upshot of this is that this one simple protein, which is pentameric which has two faces, there is an A-face and a B-face, what that protein can do really is dictated by how much CRP you make and by what's available in proximity for it to bind to. And that's really the novel thing.

You can have this one protein that can drive a lot of complex pathways that logically would be predicted to contribute to a cardiovascular disease.

Bob Barrett: So lot of information, what should be our listeners take-home message?

Alexander J. Szalai: I think the take-home message here is that while of course nature is complex, we all know that, but as I said you've got this fluid phase protein that circulates at low levels, that really acts as an adaptor, I mean it can protect against pathogens for example pneumococcus and that's a good thing but that's at some risk, I mean it can damage the endothelium, that's a bad thing. So that's one of the take-home messages.

The other is that science is never straightforward, I guess our job as researchers is to try to identify the mechanisms that underlie these clinically relevant complex diseases but what that one protein can do is equally complex. It further adds to the growing evidence that CRP matters in humans in terms of cardiovascular disease and it would strengthen in my view the argument that designing therapeutics to target, to clinically target CRP would probably – would wager, be beneficial in a clinical context.

Bob Barrett: Dr. Alex Szalai is a professor of medicine and microbiology at the University of Alabama at Birmingham and he has been our guest in this podcast from Clinical Chemistry. I'm Bob Barrett. Thanks for listening.