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Mass Spectrometry–Based Adrenal and Peripheral Venous Steroid Profiling for Subtyping Primary Aldosteronism

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Guest: Dr. Graeme Eisenhofer of the Institute of Clinical Chemistry and Dept of Medicine III at the University Hospital, Dresden, Germany; and Dr. Jacques Lenders of Dresden and the Dept of Internal Medicine Radboud University Nijmegen Medical Center in the Netherlands.

Bob Barrett: This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Primary aldosteronism, also known as Conn's syndrome, is a form of endocrine hypertension that results from excess product of aldosterone by the adrenal glands. The actions of excess aldosterone on the kidney lead to increased sodium retention and loss potassium. That retention of sodium leads to increased blood volume and hypertension, which combined with reduced serum potassium, provides the classic hallmarks for Conn's syndrome. The increased blood volume also results in suppression of rennin secretion by the kidneys as a feedback mechanism.

Differentiating patients with primary aldosteronism caused by aldosterone producing adenomas from those with adrenal hyperplasia relies on adrenal venous sampling measurements of aldosterone and cortisone. The March 2016 issues of *Clinical Chemistry* published a paper titled "Mass Spectrometry-Based Adrenal and Peripheral Venous Steroid Profiling for Subtyping Primary Aldosteronism" that examines that utility of LC tandem mass spectrometry based profiling to stratify patients with primary aldosteronism.

Two of the authors of that paper join us in this podcast. Dr. Graeme Eisenhofer is Professor and Chief of the Division of Clinical Neurochemistry at the Institute of Clinical Chemistry and Department of Medicine III at the University Hospital, Dresden, Germany. Dr. Jacques Lenders has joint appointments in Dresden and at the Department of Internal Medicine Radboud University Nijmegen Medical Center in the Netherlands.

Dr. Lenders, we'll start with you. Can you explain why adrenal venous sampling is necessary and how the procedure is performed?

Jacques Lenders: Adrenal venous sampling is necessary to identify two main forms of the disease. Most forms can result in severe hypertension and associated morbidity, but what's important

is that treatment is quite different and this requires discrimination in order to appropriately data treatment. One form, unilateral disease, is usually due to another adenoma and one adrenal gland and is best treatment by performing an adrenalectomy. This means surgical removal of the diseased adrenal gland. The other form, bilateral disease, is usually due to bilateral hyperplasia, and that's characterized by an excessive production of aldosterone by both adrenals. This form should be treated by specific drugs, mineralocorticoids receptor antagonists.

These two forms cannot adequately be distinguished by currently available imaging techniques such as CT scanning, so you need a technique that's able assess whether the excess aldosterone production is coming from one or both adrenal glands. AVS provides such technique. The procedure is carried out by inserting a catheter in the femoral vein, which is advanced under radiological guidance through the cavil vein and to the opening of both the left and right adrenal veins.

Once positioned correctly, blood is sampled for measurements of plasma aldosterone and cortisol. The measurement of cortisol serves two purposes: The first one is to confront the correct positioning of the catheters in the adrenal veins, and the second one is to normalize or correct measurements of aldosterone for differences in adrenal blood flow or for delusional effects of bloods drawn from other sources than the adrenal vein. And for a correct interpretation of the test results that entails the comparison of the ratios of aldosterone to cortisol and right versus left adrenal veins to determine whether there is unilateral or bilateral excess aldosterone production.

Bob Barrett: So this adrenal venous sampling sounds like an incredibly complex procedure that's required after an already involved laboratory work up. It seems there should be simpler procedures.

Now, Dr. Eisenhofer, perhaps you can tell us a little about this study and why you and your coauthors chose to investigate steroid profiling instead of improved or new imagine procedures?

Graeme Eisenhofer: Bob, you're absolutely correct to imply that new imaging methods would provide a simpler more direct solution. And there are certainly others working on this, including Morris Brown at Cambridge as well as Stefanie Hahner and Martin Fassnacht at Würzburg, and although I have some experience in developing PET imaging technology, my major interest actually centers around laboratory medicine. And in Dresden with Mirko Peitzsch who is our expert mass spectrometrist, we have developed mass spec based steroid

profiling, and our method here involves plasma measurements of 15 adrenal steroids, including of course aldosterone.

Now, in Europe and Germany, we are also fortunate to have great support from multi-center clinical studies with well-established networks, including the European Network for the Study of Adrenal Tumors or ENS@T. That's why where my group has taken care of the analytics, all the important patient group was carried out at three other tertiary referral centers. Munich, under the direction of Martin Reincke and Felix Beuschlein, Nijmegen under Jaap Deinum and Jacques, here, and Dusseldorf with help from Holger Willenberg, who is now with Rostock.

So with the involvement of these three centers, we were able to bring together samples from 216 patients who underwent AVS to investigate primary aldosteronism.

What was the other part of the question? Jacques, maybe you can fill in here?

Jacques Lenders: That was about complexity, Graeme, and you're quite correct, Bob, AVS is indeed a complex procedure. But on top of that, it's time-consuming and patient unfriendly. It's really a technically demanding procedure that requires the involvement of expert radiologists. So this complex and difficult procedure cannot be performed in each hospital and this is not very helpful for efficient and cost-effect diagnosis of a relatively very common form of endocrine hypertension.

Graeme Eisenhofer: Yes, that's right, Jacques. I'd like to add here that although one object in that study was to investigate whether a mass spec based steroid profiling during AVS could offer advantages over a conventional immunoassay managements, there was one other important goal as stated in the introduction of that *Clinical Chemistry* paper. That goal was to investigate whether different steroid profiles in adrenal venous plasma translates to different steroid profiles in peripheral plasma. If so, then we thought this might have significance to streamlining the diagnosis of primary aldosteronism.

Actually, the background to this approach is based on experience of both Jacques and myself in developing measurement of plasma free metanephrine for diagnosis of pheochromocytoma. The potential significance of those measurements was established from adrenal venous sampling which indicated the primary source of those metabolites, the metaneprhines from the adrenal glands as well as from tumors off that gland, that's from our experience sampling directly from the venous effluent of a

tumor can provide very valuable information about the best biomarkers to look for in systemic plasma.

Bob Barrett: You mentioned earlier, Dr. Lenders, that primary aldosteronism is common. Just how common is it?

Jacques Lenders: Well, it's much more common than previously thought. Various studies, including the groundbreaking work from Richard Gordon and Michael Stowasser at Brisbane from Australia, suggest that primary aldosteronism is responsible for anywhere between 5% and 15% of cases of hypertension, probably it's a little bit closer to the lower-end of around 5%. But nevertheless, since hypertension in the general population is quite common at about 25% to 30%, even a 5% prevalence of primary aldo among hypertensives translates into an overall prevalence of primary aldosteronism in the general population of at least 1%. Currently, the majority of patients remain, however, undiagnosed. But even if we could detect efficiently all patients with primary aldosteronism, it's quite unlikely that AVS would offer a practical solution since this would boast a big demand in terms of number of facilities able to perform adrenal venous sampling.

Given that adrenal venous sampling needs to be done at specialized centers, performing this sampling in all patients would represent a major logistic bottleneck.

Bob Barrett: How could steroid profiling help overcome these problems, or this "diagnostic bottleneck" as you call it?

Graeme Eisenhofer: Well, it's already been mentioned, what we really need are improved imaging methods, but those are probably going to be quite a long way off. And in the meantime, mass spectrometric based steroid profiling could help in several ways. First, mass spec measurements of steroid profiling during AVS offers a several advantages over conventional immunoassay measurements. The latter are less accurate and prone to assay interference. Second, cortisol is a suboptimal steroid to assist selectivity, however adrenal steroids are far more accurate.

In addition, as we've shown in our *Clinical Chemistry* paper, mass spec measurements of aldosterone provide a more sensitive method than immunoassay methods for identifying unilateral aldosterone secretion. Finally, patterns of other steroids besides aldosterone can also be used during AVS to assist in defining unilateral from bilateral disease.

For example, in another recently published study, we showed that for aldosterone producing adenomas, there were specific steroid finger prints related to the underlying

mutation. These are the somatic mutations and this can be useful in pinpointing the nature of the disease.

Now, although this might not address the bottleneck that you and Jacques mentioned, these advances should help with better distinguishing unilateral from bilateral disease.

As shown in our paper and more important to the diagnostic bottleneck, the differences in steroid profiles and adrenal venous plasma translated into distinct patterns in peripheral plasma that can be used to potentially to discriminate unilateral from bilateral disease.

Now, although AVS still remains necessary for those patients indicated to have unilateral disease, we could avoid its use in some patients, in particular the patients indicated to have bilateral disease after a needle stick, could skip AVS and go immediately into mineralocorticoid receptor blockade. Now that could reduce requirements for AVS by up to 40% or more, and this would of course alleviate to some extent that bottleneck.

Jacques Lenders: Now, we should not forget that in the future, steroid profiling using peripheral plasma could also be used in conjunction with any new imaging methods that might prove useful in distinguishing bilateral from unilateral disease, so thereby avoiding the need for AVS entirely.

Bob Barrett: Okay. Well, finally, let's talk about the future. What are the next steps in bringing the method into routine clinical practice and what kind of hiccups do you see along the way?

Jacques Lenders: I think the first thing to clarify is that our study, although it was carried down in a large cohort of patients, was retrospective and it depended on the immunoassay measurements of aldosterone and cortisol to establish the diagnosis of bilateral versus unilateral disease. What we need to do now is move into a prospective study. This is before we can even think about routine diagnostics. A prospective study is required not only to establish, whether mass spec based tier profiling also has advantages over routine measurements of cortisol and aldosterone during AVS, but also to establish whether the peripheral venous steroid profiles can in effect be used to prospectively predict bilateral versus unilateral disease, thereby minimizing requirements for AVS.

Graeme Eisenhofer: Don't forget, Jaques, that we also need to go back to basics, and establish whether in fact peripheral steroid profiles can in fact be used at the outset as a screening tool to identify among high potentsives those patients with primary aldosteronism. At that point, if we could also discriminate patients with bilateral from unilateral disease, this would

really streamline the diagnostic process. Here, we are extremely fortunate to be part of the European wide multi center headed by Maria Christina Zennaro in Paris and supported by EU HORIZON 2020 grant to establish just such an approach.

Now, as far as moving the technology into routine use, there are of course already quite a few labs using mass spec for routine measurements of aldosterone and cortisol. There shouldn't be too much of a problem for those labs to include additional steroids to cortisol, including DHEA or androstenedione, and these could be used for purposes of both calculating selectivity more accurately than with cortisol and also assessing lateralization ratios for distinguishing unilateral from bilateral aldosterone secretion.

Now, moving from mass spec based measurements in AVS studies to routine use of peripheral steroid profiles, is however entirely another ball game. It's going to probably take quite some time to bring multi steroid profiles into routine diagnosis, and this is because we are not basing diagnosis on measured values that form within or outside references intervals, but rather by how selective steroids fit within one patent versus another.

Although we can and there are established reference intervals for each of the steroids in our profile, disease stratification depends not on most defined reference intervals, but rather on mathematical constructs for relations between key steroids in the profile. Now, I envisage that this is going to take quite a while to sort out, particularly in reaching some kind of agreement in harmonization among laboratories, not to mention regulatory issues along the way. Of course, these are common problems, common issues, that are fast emerging for use of all related technologies and diagnosis. So I'm sure there will be solutions popping up in the future.

Bob Barrett: That was Dr. Graham Eisenhofer, Professor and Chief of the Division of Clinical Neurochemistry at the Institute of Clinical Chemistry and Department of Medicine at the University Hospital Dresden in Germany. He was joined by his Dutch colleague Dr. Jacques Lenders and they've been our guests in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening!