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Q&A on Pheochromocytoma

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Guests:
Dr. Matthew Estey is currently completing a Postdoctoral Fellowship in Clinical Chemistry at the University of Toronto, and Dr. Graeme Eisenhofer is Professor and Chief of the Division of Clinical Neurochemistry at the University of Dresden.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I'm Bob Barrett.

Pheochromocytomas are tumors of the adrenal gland that secrete catecholamine. Closely related tumors called extraadrenal paragangliomas can arise at extraadrenal sites. Catecholamine secretion from these tumors causes headache, perspiration, palpitations and hypertension. If not recognized and treated, pheochromocytoma and extraadrenal paraganglioma can lead to arrhythmias, myocardial infarction, stroke and death.

Diagnosis relies on biochemical evidence of excess catecholamine secretion and confirmation of tumor presence by imaging studies. While many different biochemical tests have historically been used, measurement of catecholamine breakdown products, metanephrine and normetanephrine in plasma and urine are now regarded as the first-line tests. However, it can be challenging to differentiate between true-positive and false-positive results when metanephrine or normetanephrine concentrations are only slightly above the reference limits.

The March 2013 issue of *Clinical Chemistry* includes a question and answer piece on the diagnosis, localization, and treatment of pheochromocytoma. The papers summarize the opinions of five experts representing different views on the subject.

In our podcast today, we have one of the moderators, Dr. Matthew Estey, who is currently completing a Postdoctoral Fellowship in Clinical Chemistry at the University of Toronto. And Dr. Graeme Eisenhofer, Professor and Chief of the Division of Clinical Neurochemistry at the University of Dresden.

Dr. Estey, we'll start with you. The Q&A article appearing in *Clinical Chemistry* addresses several current issues regarding testing for pheochromocytoma. Can you give us a

brief overview of the topic and what laboratorians can learn from the discussion?

Dr. Matthew Estey: Yes, absolutely. So testing for these tumors can be divided into three different categories. The first is biochemical testing, and this looks for an evidence of excess catecholamine production, and these are simply hormones that are naturally released by the body. Measurement of metanephrines, which are breakdown products of these catecholamines, are regarded as the best first-line test. However, these molecules can be measured in either plasma or in urine, and it remains unclear which of these tests is the best test. It can also be quite challenging to distinguish false-positive from true-positive results when these metanephrines are only slightly increased.

The second category is imaging tests and these are used to localize the tumor. CT, MRI and various different types of functional imaging studies are all commonly employed.

The last category is genetic testing. The number of pheochromocytoma susceptibility genes continues to grow. And at least 30% of these tumors are now known to be hereditary. Importantly, genotype-phenotype correlations have been elucidated, so identifying the affected gene in a given patient is important for both patient management and for testing of those patient's relatives. However, the best way to go about approaching the genetic testing in a given patient with pheochromocytoma remains a very important issue.

So in our Q&A article, five experts from around the world described their approach to pheochromocytoma testing, and they also provided their insight on to many of these outstanding issues. They also discussed the relevance of these tumors and the treatment options for patients who are diagnosed with pheochromocytoma.

Bob Barrett: Thank you, Dr. Estey.

Now, Dr. Eisenhofer, several different tests have been used over the years to screen for pheochromocytoma and paraganglioma, but plasma and urinary metanephrines are now regarded as the best first-line test. What exactly are metanephrines and why are these tests superior over others?

Dr. G. Eisenhofer: The metanephrines are isolated metabolites of catecholamines. Normetanephrine is the metabolite of norepinephrine or noradrenaline. Metanephrine, the metabolite of epinephrine, and methoxetamine is the metabolite of dopamine. Normally, methoxetamine is not

considered as a part of the metanephrines, but we'll consider this part of the metanephrines here.

These metabolites are produced in relatively low amounts compared to the deaminated metabolites. The single largest of source of the metabolites is the chromaffin cells of the adrenal medulla. In fact, the chromaffin cells of the adrenal medulla normally account for 91% of all circulating metanephrine. And this makes the metabolites superior to any other metabolites for diagnosis. But more importantly, the metabolites are produced continuously within adrenal chromaffin cells by metabolic processes that are independent of catecholamine release. Catecholamines that were normally measured for diagnosis with this continuous production makes the metanephrines a better biomarker than the catecholamines which can be secreted intermittently or in low amounts.

Bob Barrett: Metanephrines can be measured in plasma and urine. Are there differences in these measurements?

Dr. G. Eisenhofer: Yes. Normally in urine they are measured after an acid hydrolysis dip which cleaves the sulfates, so they represent both free and conjugated metabolites in urine.

Whereas in plasma, they are typically measured without a deconjugated step, so they're measured normally as free metanephrines. But in actual fact, they can be measured as either the deconjugated or free in either matrix, but it's the free that are mainly measured in plasma and the deconjugated in urine.

Bob Barrett: Are there any important differences between measurements as free or deconjugated metabolites?

Dr. G. Eisenhofer: Yeah, they're different metabolites. The free metabolites produced by catechol-O-methyltransferase that's the enzyme in the chromaffin cells, and they're rapidly cleared from the circulation. So they have very short plasma half-lives and as a consequent, they are present in plasma at very low concentrations.

The sulfate conjugates, on the other hand, downstream metabolites, they're produced by an enzyme located principally in the gastrointestinal tract, and they have slow clearance. They're principally cleared by the kidney so that they're present in plasma in much higher concentrations. They're also of course the main form that's excreted in the urine.

Bob Barrett: Do these differences provide an advantage for measurements of one over the other form of these

metabolites, and which test or tests would you recommend and why?

Dr. G. Eisenhofer: Well, in theory for diagnosis, the plasma test should be better. This is because of the significant amount of the normetanephrine sulfate, is formed from norepinephrine that's originally produced within the gastrointestinal tissues. And these tissues are a major, major source of all norepinephrine produced in the body. About 40% of all norepinephrine produced in the body is produced in the GI tract. But most of the free norepinephrine and free normetanephrine produced in the GI tract is metabolized in the liver. It doesn't get through to the systemic circulation.

In contrast, the sulfate conjugates do, and therefore there are larger proportional increases in the free normetanephrine than the sulfate conjugates of normetanephrine in systemic plasma. It's a biomarker of secretion by the pheochromocytomas.

This is all in theory, but in practice the differences are not very well established and there remains controversy over the relative advantages and disadvantages of either plasma or urine tests. Both of course therefore remain recommended for diagnosis. Nevertheless, I would recommend measurements of the plasma free over the urinary deconjugated metanephrines, but only when available methods have sufficient analytical sensitivity for accurate measurements of the very low levels in plasma. Otherwise, urinary measurements are fine.

But, here, I wonder why we are still measuring routinely the deconjugated metabolites because with modern techniques the analytical sensitivity is more than sufficient to measure the free metabolites, and this really avoids need for an acid hydrolysis step and this is poorly controlled for since there are no appropriate calibrators or QC samples for the sulfate conjugates. And I think this certainly provides a practical advantage over measurements of the free versus the deconjugated metanephrines in urine.

Bob Barrett: Biochemical markers can typically be measured using different technologies. What measurement methods are available for metanephrines?

Dr. G. Eisenhofer: Well, today, there are three main techniques. There is HPLC with electrochemical or coulometric detection. There is also liquid chromatography with tandem mass spectrometry. And particularly in Europe and other countries, there are immunoassays, but these assays are not widely used in the U.S.

Bob Barrett: So, Doctor, what are the advantages and disadvantages of the three measurement methods?

Dr. G. Eisenhofer: Well, up until recently, my experience has mainly been with liquid chromatography with electrochemical detection. This is the method we started with. When I arrived in Germany, the assays here were all by immunoassay, and it's only recently that we have moved to mass spectrometry. I can certainly say the advantages of the immunoassays, which are available in kit form, makes them easily adaptable to analyzer instrumentation, and this requires minimal expertise and very low start-up costs. However, this method suffers from calibration problems, they're inaccurate and not very precise, and it is now clear from inter-laboratory proficiency programs that they certainly suffer compared to mass spec and the electrochemical detection methods. They also don't allow measurements of methoxetamine. Not as huge anyhow.

LCECD that's with electrochemical detection. Now, this is an okay method and reasonably accurate, but it's cumbersome and it's really a difficult method. Now that we've moved to mass spec, I can see enormous advantages of this technique. The only disadvantage, of course, is the relatively high cost of the instrumentation and the need for expertise to run the machines.

But the methods have high throughput. The consumables are relatively low cost particularly compared to the immunoassays. These assays have high analytical specificity with a lot more freedom from interferences from the other techniques, and as well as this with the modern instruments, the analytical sensitivity is actually far superior than with LCECD. So really, I far prefer mass spectrometry over the other methods.

Bob Barrett: Doctor, inadequate patient preparation for blood sampling is a widespread problem in the workup of potential pheochromocytomas and paraganglioma cases. What are ramifications of the improper patient preparation and how can these be prevented?

Dr. G. Eisenhofer: The ramifications that patients aren't properly prepared are false-positive results and this is a major problem because these tumors are rare, but they have to be tested in a large population. So typically the false-positive results overwhelm true-positive results. This is a big problem, and typically, it's caused by inadequate preparation of the patients. These are metabolites of catecholamines and the catecholamines are stress hormones, and the metanephrines are rapidly cleared and they also respond to stress.

So the patients have to be adequately prepared at least for the blood test. There should not be any form of stress, and stress includes upright posture. So the sampling should be done in the supine position, and typically, it's not. Typically, it's done in a seated position. This is wrong. Levels of normetanephrine in particular, on average 30% higher in the seated than supine position, but they can be much higher than that in some individuals.

Seated sampling is preferable because it's easier. But if it's done and it's likely to return false-positive results, the clinicians must be aware of this if they're going to do the sampling in a seated position. They must be prepared to repeat the sampling in the supine position with 30 minutes of supine rest.

I think, really, the prevention ultimately requires improved education of clinicians. There are other factors that can help and increase specificity problems, reduce false-positives. Reference intervals are important to set up appropriately. This can be set up according to age. The normetanephrine increases with age, so age-appropriate reference intervals can help with increasing specificity and reducing false-positive results.

Bob Barrett: In instances where plasma or urinary metanephrines are slightly or modestly increased, what additional tests are useful in investigating a potential case of pheochromocytoma?

Dr. G. Eisenhofer: First, as I've just mentioned, you have to consider causes of false-positive test results. Was the sampling carried out under stress situations? Was the patient seated instead of being supine? Medications can also be an issue, tricyclics. So all these factors should be considered. The test should be repeated, taking into account these factors and particularly excluding these factors. After that, then follow-up tests should be considered, and in here, one needs to consider that these follow-up tests should be just as diagnostically sensitive as the initial screening test, but preferably more specific, more diagnostically specific than that screening test. This means of course that a positive test for urinary fractionated metanephrines is best followed up with a plasma test, which when carried out correctly provides higher diagnostic specificity than the urine test.

Now, for repeated slight elevations of normetanephrine, in plasma at least, the clonidine suppression test is also useful, and this can be performed with measurements of plasma normetanephrine before and three hours after oral clonidine. A wait and retest approach can also be useful to check for continuing elevations. This is particularly important for metanephrines. When metanephrine only is increased, you

cannot use the clonidine test with metanephrine only with normetanephrine, so a wait and retest approach is useful there. But all of these depends on the initial level of clinical suspicion and the presentation of the patient, which is really over to the clinician.

Bob Barrett: It's been estimated that at least 30% of pheochromocytomas and paragangliomas are hereditary. Would you therefore recommend genetic testing in all patients with the tumors?

Dr. G. Eisenhofer: Well, it's generally recommended that genetic testing should be considered in all patients with the tumors, and certainly, I endorse that recommendation. But on the other hand, this does not mean that genetic testing should be done on all patients with tumors. This is particularly important because there are now 10 tumor susceptibility genes. Actually last week there was an eleventh gene identified. So there are many, many tumor susceptibility genes to consider and testing of these genes is not cheap. At the moment, it's very expensive.

So, I think the testing has to consider all kinds of other variables. The presentation of the patient and other things.

Bob Barrett: Well, under what circumstances would you recommend genetic testing?

Dr. G. Eisenhofer: Well, there were several groups where genetic testing should be -- well, first of all, any patient with additional tumors or clinical sign stigmata that would indicate a hereditary condition, making point to a specific gene to test. Family history of paraganglioma also means that testing should be carried out. If the patient has bilateral adrenal pheochromocytomas or multifocal disease, this almost always indicates an underlying hereditary cause. Age of diagnosis is also important to consider. Any patient below the age of 30, particularly children, they should be tested for mutations. Extraadrenal tumor location is another factor, and the presence of metastatic disease.

But even amongst all these groups, I would recommend testing of every gene. Certainly, however, once the technology is available and the cost comes down of highest throughput screening, then this might be possible. But at the moment it's not possible.

Bob Barrett: Well, until that time, which genes would you recommend testing?

Dr. G. Eisenhofer: Well, as I have mentioned earlier for patients with clinical stigmata there are specific signs or conditions that can point to a specific gene. In Von Hippel–Lindau Syndrome, the

presence of retinal hemangioblastomas are common, so the presence of these tumors would mandate testing of the VHL gene. The patients without any stigmata, but with a family history, for instance, young age or bilateral multifocal disease, testing should be guided by the biochemical profiles. Biochemical profiles vary distinctly depending on the underlying mutation. Patients with elevations of metanephrines -- this is the adrenaline metabolite -- this would indicate testing for the RET proto-oncogene or the TMT-TMEM127 gene, patients with only increases on normetanephrine. This mandates testing of the VHL gene and SDH genes and with elevations of methoxytyramine, the SDH genes. These are the genes, the subunits of succinate dehydrogenase.

But even with all of these, these too take into account other factors which can override and be of even more importance than the biochemical profile. Tumor location is important. If they're adrenal tumors, this suggest that VHL and RET mutation testing are more important than SDH testing. On the other hand, if they're extraadrenal tumors, testing of the SDH genes is most important. And if the patient presents with metastatic disease then this mandates SDHB mutation testing. Patients with SDHB mutations have a high risk of malignant disease. Beyond this immunohistochemical analysis of tumor tissue for SDHB staining can also be useful to guide testing of these genes.

Bob Barrett: Well, finally, doctor, your laboratory has recently published several studies suggesting that methoxytyramine is a useful marker in evaluating cases of pheochromocytomas and paraganglioma. What is the utility of this novel marker and how is it measured? And do you anticipate methoxytyramine will become a standard test?

Dr. G. Eisenhofer: Methoxytyramine is present in plasma in an extremely low level, so it’s difficult to measure accurately. But with new mass spectrometers it can measured, and we have found that elevations of this metabolite typically occur in patients with mutations of SDHB and SDHD genes. They often occur with patients with paraganglioma as extraadrenal tumor not adrenal tumors. And we’re also seeing that this metabolite can be a pretty good biomarker for metastatic disease. So any patient with an elevation of methoxytyramine, one needs to consider that patient to have either metastatic disease or there could be an underlying mutation of SDH genes. My personal opinion is that it's best to measure this metabolite by mass spectrometry, as I've said, since the levels are very low and only the new mass spectrometers have sufficient analytical sensitivity for these measurements.

Now, initially, we thought that these measurements should only be restricted to screening patients with SDH mutations, but now with the mass spec results, I'm starting to rethink this, and maybe these measurements should be applied generally a part of testing and that they can be easily measured as part of the typical test of normetanephrine and metanephrine. It could be added to this test.

Methoxytyramine can also be measured in urine, but we have yet to determine what really the urinary levels indicate. And there is major word of caution with methoxytyramine. It's susceptible to many dietary influences. So when you sample blood for measurements of methoxytyramine, it is an absolute must that sampling must be carried out after an overnight fast. And this also might favor the plasma test over the urine test but we have yet to determine that. Time will tell.

Bob Barrett: Dr. Matthew Estey is currently completing a Postdoctoral Fellowship in Clinical Chemistry at the University of Toronto, and Dr. Graeme Eisenhofer is Professor and Chief of the Division of Clinical Neurochemistry at the University of Dresden. They have both in our guests in this podcast from Clinical Chemistry. I'm Bob Barrett. Thanks for listening.