Clinical Chemistry

# Plasma Steroid Metabolome Profiling for Diagnosis and Subtyping Patients with Cushing Syndrome

**Article:**
Graeme Eisenhofer, et al.
*Plasma Steroid Metabolome Profiling for Diagnosis and Subtyping Patients with Cushing Syndrome.*
Clin Chem 2018;64:586-96.
http://clinchem.aaccjnls.org/content/64/3/586

**Guest:** Dr. Graeme Eisenhofer is professor and the Chief of the Division of Clinical Neurochemistry at the Institute for Clinical Chemistry and Laboratory Medicine at the Technical University in Dresden, Germany.

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.

Just over 100 years ago, a neurosurgeon at Johns Hopkins reported a study on a condition caused by a malfunction of the pituitary gland which he termed "polyglandular syndrome." However, that physician, Harvey Cushing, has had his own name associated with that disease ever since. Cushing's syndrome can result from a number of causes, but most usually involves small pituitary tumors that secrete ACTH which results in an increase production and release of cortisol by the cortex of the adrenal gland.

As might be expected, diagnosis of this disease depends on laboratory measurements of cortisone. However, the use of plasma or urinary multi-steroid profiles for better characterizing patients with Cushing's syndrome had been suggested and distinct steroid profiles may be useful in the differential diagnosis.

A paper in the March (2018) issue of *Clinical Chemistry* describes a liquid chromatography tandem mass spectrometry procedure for multi-steroid profiling in Cushing syndrome and other disorders. The lead author of that study is Dr. Graeme Eisenhofer, who is professor and the Chief of the Division of Clinical Neurochemistry at the Institute for Clinical Chemistry and Laboratory Medicine at the Technical University in Dresden, Germany. He is our guest in this podcast.

Doctor, what advantages are offered by measuring a panel of steroids, the steroid metabolome, as you call it in your paper compared to already routinely available single measurements of cortisol?

Graeme Eisenhofer: Well, that's not a simple question to address particularly since there is much more to the diagnosis of Cushing's than simply measuring cortisol; nevertheless, the short answer

that the steroid panel offers a single test alternative for both initial screening and subtype classification.

Currently the diagnosis of Cushing's is an arduous, multi-step process. The first screening step typically involves multiple tests, 24-hour urine-free cortisol, salivary-free cortisol and the low dose dexamethasone suppression test. All are inaccurate and show a considerable overlap between patients with or without disease.

Consequently, the tests often have to be applied in combination and on multiple occasions. However, that is only the start of the diagnostic process. Once hypercortisolism is diagnosed the particular subtype has to be identified. There are ACTH dependent and independent subtypes. The former are usually due to the classic pituitary adenomas first described by Harvey Cushing. In the latter, adrenal tumors that directly produce cortisol.

Now, the two subtypes are usually differentiated by additional measurements of ACTH with obviously high levels of ACTH in the ACTH dependent subtype and low levels in the independent subtype that's that adrenal form, due to feedback inhibition by cortisol produced by the tumor.

In ACTH dependent Cushing's there can be additional ectopic subtypes where the ACTH is not produced by the pituitary tumors but rather by tumors at other locations such as the lungs.

Distinguishing ectopic in pituitary subtypes is particularly difficult and requires numerous dynamic and often invasive tests, high dose dexamethasone suppression test, the CRA stimulation test, petrosal sinus sampling with ACTH measurements, to name just a few.

Measurements of the steroid metabolome although I'm lightly to circumvent the use of all these currently applied tests, they nevertheless can be employed as a single initial test with potential for streamlining the diagnostic processes by helping to more effectively confirm and exclude disease. And at the same time, pointing clinicians in the right direction for more rapid classification of the particular Cushing subtype. That really is the main advantage that the steroid metabolome has to offer over currently available screening test using cortisol.

Bob Barrett: In your study, you excluded patients without a diagnosis. Does that risk introducing some bias into the study?

Graeme Eisenhofer: Yes, and that's the limitation of the study. Patients with a positive result for the dexamethasone suppression test were excluded if we could not positively confirm disease or

exclude disease by follow up. Without a diagnosis, we have no way of knowing whether the steroid metabolome might have been useful in those patients who were excluded from the study.

This also means that we could not determine whether the test actually offered any diagnostic advantage over routine screening test. We could therefore essentially only determine whether the new test was good as the routine test. And this is actually a common problem to many retrospective diagnostic studies.

There is no easy way around the problem without a different prospect of design to the study and long-term follow up with some alternative method to exclude disease.

Bob Barrett: Are there any other advantages of the panel that your group is proposing?

Graeme Eisenhofer: Well, the test as examined can be employed from a single blood sample drawn in the morning as it is most usual for outpatient visits. This is compared to standard tests that employ inconvenient 24-hour urine collections, midnight salivary collections for free cortisol, or the dexamethasone suppression test.

Bob Barrett: What about simply measuring just plasma cortisol? Isn't that part of the panel already?

Graeme Eisenhofer: Yes, it's part of the panel, but morning or random cortisol is well-established to be next to useless and is not recommended. In our study, it showed considerable overlap and even the deoxycortisol is much better than cortisol was a single analyte.

Now, the reason for this is partly because cortisol undergoes pronounced diurnal changes with highest values in the morning and lowest late at night. The nadir late at night is when it is best to measure cortisol, to take disruption to the diurnal changes and that's a signal from a tumor.

Cortisol and plasma is also heavily protein-bound which also posess additional problems, hence measurements of midnight salivary-free cortisol or integrated 24-hour urine-free cortisol.

Bob Barrett: Do the other steroids in the panel undergo similar diurnal changes?

Graeme Eisenhofer: Yes, as documented by the group of Steve Soldin in a previous issue of *Clinical Chemistry*. Many other adrenal steroids do show similar diurnal changes to cortisol.

Bob Barrett: So, these most make an important to use reference intervals set up for the specific times during the day at which samples are taken for analysis?

Graeme Eisenhofer: Indeed, that's correct. Reference intervals must match to the time of sampling. However, it actually gets more complicated than this since there are also highly dynamic changes in steroids with aging. And these increase from childhood and then steadily decreased after the early 20s. There are also marked gender differences need to be considered. So, in our analysis we used age and gender specific reference intervals.

Bob Barrett: Isn't the need for aging gender specific reference intervals another disadvantage for steroids metabolome test?

Graeme Eisenhofer: Well, it certainly complicates things but I wouldn't call it a disadvantage. These days, as long requests are accompanied by appropriate patient information, it is really relatively a simple matter to program reporting of results with age and gender specific reference intervals. The only real problem is establishing or validating those age and gender specific reference intervals in the first place. However, once established the advantages and better defining results that fall within or outside of normal limits thereby increasing diagnostic accuracy.

Bob Barrett: Well, finally doctor, are there any other hurdles that need to be met and looking ahead, what are the next steps to bringing steroid metabolome diagnostics to the routine laboratory?

Graeme Eisenhofer: Well, gaining clinical acceptance will be key, and for that we need carefully design prospect of studies. Harmonization between laboratories is also a problem that needs to be addressed. We also have to move from unidimensional thinking to a multidimensional approach in how we interpret and present test results that involve panels of analytes.

One other thing, I forgot to mention one very important advantage before, of the steroid metabolome panel. It is a test that can be applied to other disorders of adrenal steroid function. For example, we use exactly the same panel to diagnose primary aldosteronism. And other at the Mayo Clinic and King's College in London have recently described in this past November and December issue in the *Clinical Chemistry* applications of steroid panels for adrenal cortical carcinoma.

So, this is another adrenal disorder such panels can be applied for. But there are many more possibilities with further modifications such single test panels can be applied to the diagnosis of multiple disorders. And this is already

 

being done within metabolomics based diagnostics. So, there should be no reason why we cannot do the same thing with mass spectrometry.

Bob Barrett: That was Professor Graeme Eisenhofer from the Institute for Clinical Chemistry and Laboratory Medicine at the Technical University in Dresden, Germany. He's been our guest in this podcast on the steroid metabolome. I'm Bob Barrett.