High-Throughput LC-MS/MS Method for Determination of the Alcohol Use Biomarker Phosphatidylethanol in Clinical Samples by Use of a Simple Automated Extraction Procedure—Preanalytical and Analytical Conditions

Article:
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Guest: Dr. Anders Isaksson is assistant professor in the Department of Laboratory Medicine in the Division of Clinical Chemistry at Lund University in Lund, Sweden.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk" from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

Alcohol use disorders are a major health problem worldwide and alcohol biomarkers are an important tool for diagnosis, follow-up, and treatment. Recent alcohol use can be evaluated by measuring ethanol in serum, urine, or breath or through the analysis of urine ethyl glucuronide or ethyl sulfate. More long-term use has conventionally been evaluated through measurement of carbohydrate deficient transferrin or gamma glutamyl transferase. Recently, phosphatidylethanol has emerged as the more sensitive and specific marker of alcohol consumption, but a high capacity, reliable, and cost effective method was lacking.

A high throughput LC-MS/MS method for the determination of phosphatidylethanol (PEth) in clinical samples using a simple automated extraction procedure was published in the May 2018 issue of *The Journal of Applied Laboratory Medicine*. This work describes an improved automated approach to analysis of the alcohol marker phosphatidylethanol that uses liquid chromatography mass spectrometry instead of conventional HPLC.

The first author is Dr. Anders Isaksson. Dr. Isaksson is assistant professor in the Department of Laboratory Medicine in the Division of Clinical Chemistry at Lund University in Lund, Sweden. He is our guest for today's podcast. Welcome, Dr. Isaksson. What is phosphatidylethanol (PEth) and how is it formed?

Anders Isaksson: Well, phosphatidylethanol, in short PEth, is an abnormal phospholipid only formed in the presence of ethanol. This molecule was discovered in 1983 by Professor Christer Alling, a former colleague of mine. He and his coworkers were investigating the effects of ethanol on the composition and concentration of phospholipids in different tissues and to do that, they fed rats with ethanol. On a thin layer

chromatography, they found an extra spot in lipid extract formed tissues of ethanol-fed rats.

Later, they showed that this molecule was PEth. Phosphatidylcholine is a precursor of PEth and that is formed from its precursor through the action of the enzyme phospholipase D. Phosphatidylcholine is an important component of the cell membrane, so this conversion of the phosphatidylcholine to PEth takes place within the cell membrane.

Randye Kaye: I see, thank you, and why is PEth a good alcohol marker?

Anders Isaksson: Well because for one reason is that it can be measured in blood as PEth is incorporated in the cell membrane of the red blood cell. Interestingly, this applies only to humans. No PEth has been found in erythrocytes from other investigated species. Now, the reason is that it has high clinical sensitivity and specificity since it is an alcohol metabolite, PEth correlates with the alcohol consumption level. It has a long window of detection, approximately the same as carbohydrate-deficient transferrin, with a short name CDT, which is a commonly used alcohol marker. The long window of detection is because the half-life of PEth in circulation is quite long, about one week.

Randye Kaye: Thank you. Now, can you give me a short description, can you describe your method for determination of PEth?

Anders Isaksson: It's simple. Blood is drawn in a tube with EDTA as anticoagulant. The sample workup is largely robotized and consists of protein precipitation with isopropanol. Off the centrifugation, the supernatant is injected on to the column, which is C18 reverse phase column. Therapist then quantified where a liquid chromatographic-tandem mass spectrometry method we use to direct in to this tandem.

The method has the high capacity and this is due to its short turnaround time where a new sample is injected every 30 minutes. That, similar to its precursor, phosphatidylcholine, contains two fatty acids. These can vary which means that there are a large number of different molecular forms of PEth. We determined a form that contains one palmitic and one folic acid residue as it is usually found in the highest concentration.

Randye Kaye: So, how long has PEth has been used as an alcohol marker and has the number of requests changed over time?

Anders Isaksson: Our laboratory started with PEth as a clinical alcohol marker in the beginning of 2006. It was however not until 2010 that other laboratories in Sweden began to perform PEth analysis and now there are a lot of laboratories doing it. I

think that PEth has also begun to be used in some other Nordic countries and perhaps in some other countries in continental Europe. The number of requests for PEth analysis at our laboratory has increased almost exponentially from 1,300 for the first year to over 60,000 last year. For comparison, we perform approximately four times as many PEth analysis as CDT analysis.

Randye Kaye: Right, very interesting, so my final question to you, doctor, is how stable is PEth during storage or transport and are there any other pre-analytic factors to take into account?

Anders Isaksson: It is stable for a week at room temperature and for at least three weeks in refrigerator. At minus 80 degrees Celsius, it is stable for at least two years. However, the PEth can be raised in vitro in samples containing ethanol. This information is low and clinically insignificant in EDTA samples of the storage at the room temperature for up to two days and that is even in the presence of high ethanol concentrations.

PEth can also be formed in vitro below zero so samples should not be stored in a regular freezer at minus 20 degrees Celsius. For long-term storage, PEth should be stored at minus 80 degrees Celsius. At this temperature, PEth is stable and there is no in vitro formation. Another option would be to use dry blood spots as sample material. Under this condition, PEth is stable up to a month at room temperature and no formation of PEth can occur under these conditions.

Randye Kaye: Thank you very, very much. That was very interesting. Thank you for joining me today.

Anders Isaksson: Thank you for the opportunity.

Randye Kaye: That was Dr. Anders Isaksson, assistant professor in the Department of Laboratory Medicine at Lund University, talking about a high throughput LC-MS/MS method for the determination of phosphatidylethanol PEth in clinical samples using a simple automated extraction from the May 2018 issue of JALM.

Thanks for tuning in for “JALM Talk.” See you next time and don’t forget to submit something for us to talk about.