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From DESI to the MasSpec Pen: Ambient Ionization Mass Spectrometry for Tissue Analysis and Intrasurgical Cancer Diagnosis

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Guest: Dr. Valentina Pirro is a research scientist in the chemistry department at Purdue University.

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.

Whether alone or often in combination with other therapies, surgery is a common form of treatment in patients with solid tumors. Depending on the cancer, surgery may be used to remove cancer that is contained within a specific area, to debulk a tumor, or to ease symptoms caused by a tumor. In each case, it is critical to distinguish cancerous from noncancerous tissue during the operation.

Today, pathologists perform rapid microscopic investigations of biopsied tissue and make the diagnosis. In the future, this may be conducted using mass spectrometry based measurements. Ambient ionization mass spectrometry methods make localized molecular level chemical analysis of tissue samples possible at atmospheric pressure and without prior sample purification and pre-treatment.

Over the past decades, scientific advances have extended the role of these methods from the research laboratory to the operating room. One of most exciting applications is in the direct and real time diagnosis of potentially cancerous tissue during surgery. Additional studies will follow, but the latest iteration of ambient ionization mass spectrometry, the MasSPec Pen shows promise in overcoming barriers of in vivo and of non-destructive molecular tissue analysis.

The April 2018 issue of *Clinical Chemistry* includes a commentary on the different technologies and their potential in cancer diagnosis. We're joined by Valentina Pirro, an author of the commentary. She is a research scientist in the chemistry department of Purdue University, working with Dr. Graham Cooks on development of cutting edge mass spectrometry strategies for point-of-care testing.

So, Dr. Pirro, can you briefly introduce what ambient ionization mass spectrometry is, and how many techniques have been developed so far?

Valentina Pirro: Yes, so desorption electrospray ionization was the first ambient ionization method that was developed almost 10 years ago and today, there are about 80 methods that have been described in the literature but many of them are very similar to each other and they can certainly be grouped under the same umbrella.

There is a nice review that was recently published in *Analytical Methods* and it describes the different ionization strategies so, I recommend reading it if of interest.

The general idea is to create a system that allows for generation of ions at atmospheric conditions. In other words, you are creating ions outside the vacuum system of the mass spectrometer with minimal sample preparation. So, an ambient method allows you to have a complex sample in proximity of the mass spectrometer, let's say, a piece of human brain tissue, perform ionization and mass analysis in real time, and it's this immediacy of sample preparation that differentiates ambient ionization methods from other direct mass spectrometry methodologies.

The current trend is to design methods that integrate sampling and ionization steps together, and so methods like REIMS or MasSpec Pen that was recently presented try to combine these steps together and the idea is to further streamline the analysis, and because the goal is to have no sample preparation, no sample handling, real time analysis, these methods are prone to point-of-care testing.

Bob Barrett: And how has this technology been used in healthcare?

Valentina Pirro: Right now, the biggest application we are trying to investigate is surgical margins assessment for cancer diagnostics. The general idea is that tissue analysis using mass spectrometry can show you a different chemistry between different tissue types. So, let's say a piece of normal tissue or a piece of tumor tissue, and if this differentiation is highly discriminant, you can use the chemical profiling for cancer diagnostics.

Because these ambient ionization methods give you the means to analyze the sample in a rapid and straightforward way, we can do bench to bedside translation. So, in other words, so you can bring the mass spectrometer to the sample, let's say, a piece of tissue, while you are doing surgical resection, rather than taking the sample to the laboratory for testing. In the case of cancer diagnostics, this is what the standard of care is, a piece of tissue is taken, sent to a pathology lab to do histopathology and genetic testing, and the turnaround is even 30 minutes or an hour or for genetic testing it is even days and weeks.

So, the moment you have the instrument in proximity to the surgical field and the chemical analysis of the tissue which is diagnostic can be done within seconds, then you have a possibility to give real time pathological feedback to a surgeon and the surgeon can use the information to guide a surgical tumor resection. Let's say you could map the resection margins and assure that total tumor resection is being achieved and so, you don't have residual tumor.

Because histopathology is time consuming, this is not typically done. So typically surgical margins are not analyzed or at best, they are analyze post-operatively and so, if they find a positive margin, then a patient may have to undergo surgery again. The only techniques that are used intra-operatively for this type of purpose, so assessment of surgical margins, are essentially MRI and fluorescence imaging and they help to visualize the tumor, help guide in tumor resection, but they also have limitations in the pathological information that they give and so, we want to complement that by implementing this ambient mass methods in healthcare system.

Bob Barrett: You can do that all right there during surgery, that's something.

Valentina Pirro: Yes, so there have been several methods that had been developed in which the instrument is just a couple of feet away from the surgeon and as the tissue is resected, you can do detection of metabolites, detection of lipids that are known to be altered when you have cancer and therefore, you can provide a diagnostic feedback. It's pretty impressive, certainly, a nonconventional laboratory setting.

Bob Barrett: So that your work focus is specifically on brain cancers. What are the unique challenges of brain cancer diagnosis and how did they effect the development of mass spectrometry-based diagnostic methods?

Dr. Valentina Pirro: Yes, we worked with gliomas mostly, which are malignant brain tumors. So the problem with brain cancers like gliomas is that they tend to infiltrate very deeply into the brain and so, they officially have no tumor borders, which poses a challenge for the surgical resection. What is known for brain cancers like gliomas is that no complete surgical resection can be achieved, essentially because the infiltration is way too deep into the brain.

And so what neurosurgeons need to do is to understand how they can maximize the tumor resection while minimizing the post-operative neurological deficits, and so, they need to understand how much brain matter is best and safest to cut, and there are other surgeries in which the tissue can be resected way beyond the tumor borders just to be sure that

you have negative margins, and maybe the problem is more cosmetic, but this is not the case for brain tumors especially when you have tumors that are growing very closely to areas that are important, vital, and cannot be touched.

So for us, this means that when we do our cancer diagnostics based on mass spectrometry, we are not trying to distinguish between what is normal and what is tumor to make sure that we have negative margins, so you don't see tumor anymore, but what try to do is to estimate the tumor infiltration, so give outputs like the tissue is infiltrated 20, 50, 90 percent and this is a deeper level of information that you are trying to get from the chemistry of the cells from the mass spec output. And so right now, we are understanding how much accurately and precise we can give this type of feedback.

This officially means that when we do our analysis, the workup of the data is different and instead of using classification systems like other articles that had been, techniques that are being publish, then we try to develop progression models.

Bob Barrett: How do you translate the measurement of a full mass spectrum into a normal versus tumor output?

Dr. Valentina Pirro: So, essentially what we typically collect is full scan mass spectra. So, you can see a profile of molecules that are expressed in this spectrum, and what is diagnostic can be either the presence or the absence of certain peaks of certain molecules or you can simply have changes in their relative intensity, so they can be over expressed or under expressed relatively to each other.

So, the typical workflow is that you have, you analyze the known tissue types, a piece of normal, piece of tumor tissue and you create a library of this mass spectra to build the classification system. So they essentially end up being fingerprints, bar codes of the tissue, and then you use algorithms that are based on pattern of recognition to match the mass spectrum of an unknown tissue to those of the tissue types that you include in your library so you can call for a diagnosis which you know, using computers and software, you can easily translate into a color coded diagnosis for example.

So, if you are analyzing a piece of tissue, you can color code red, the diagnosis of tumor or green, the diagnosis of normal tissue, and so you have an easy output that a surgeon can interpret even though he might not know the way the analysis or the way the mass spectrometer is working up the data. Essentially, the logic is the same

behind EI libraries and algorithms for structural identification of unknown compounds.

Bob Barrett: In the commentary, you talked about the existence of online and offline assays for tissue analysis. Can you explain what online and offline means in the context of using ambient ionization mass spectrometry for intra-surgical cancer diagnosis, and are there any benefits of one type over the other?

Dr. Valentina Pirro: Yes. So, okay, online methods aim to use a probe that can be handled by the surgeon directly to sample tissue in vivo and they typically tend to want to have real time mass spectrometry feedbacks. So methods like REIMS or the MasSpec Pen have a transfer line that connect from the probes to the mass spectrometer for immediate analysis and no sample handling whatsoever is necessary. The delay between the sampling of the tissue to the MS analysis is just a few seconds typically and the offline methods instead work around the concept of resecting a biopsy without interfering with surgical procedures.

So, essentially without having the surgeon use a different type of probe or a specialized the probe to do the sampling, and then the tissue is analyzed ex vivo in proximity to the surgical field and the methods typically have a turnaround of a few minutes.

So, there are benefits and limitations in both, and this is just my personal opinion of course. The online methods are really promising and probably the way to go to develop intra-surgical MS technology because the immediate feedback is really what you want when you want a guide a surgical resection and map surgical margins, right?

However, I have been working on developing an intra-surgically mass method for 3 years and I see how much of the surgical procedures have changed from patient to patient and from surgeon to surgeon and so, it is difficult to imagine that our unique probed design for in vivo tissue sampling could work in all circumstances, or that different designs can be made to adapt to different situations without significantly changing your analysis performances.

Another limitation I've foreseen is the optimization of the method itself. So, when you are trying to in vivo nondestructive biocompatible analysis, then you are very limited in the conditions that you can use to do so. Let's take the example of the MasSpec Pen. The MasSpec Pen relies on a direct contact between the tissue and a liquid and so, the liquid can be either sterile water or irrigation solutions. The solvent you are using to extract the molecules for diagnosis is a delicate parameter to be

optimized and you just don't have a lot of flexibility, a lot of freedom in changing your conditions to make sure that you have the best of data quality, enough sensitivity et cetera.

On the other hand, the offline methods are cumbersome and laborious compared to an online method. You still need to have personnel, a technician that would take the sample, prepare it, move it into the instrument, run it and the turnaround is longer, it's minutes. So, this, if you want to do one sample run, one sample after another, they can become complicated they can become chaotic and essentially defeat the purpose of rapid intra-operative pathology.

At the same time, you have a physical piece of tissue, you are not into the sterile surgical field anymore and so, you can optimize your analytical method to target for specific analytes, to get more chemistry and to just optimize your conditions on a deeper level. This is the first thing I would say, that make me think about pros and cons of both, and I really think that in the future, the cost, the automation of the technology, the routine use experience will drive towards either one of those techniques.

Bob Barrett: Well, finally, let's talk about the future. Where do you see this research in 5, maybe 10 years? What are the challenges you think you'll be facing?

Valentina Pirro: I really hope that in 5 or 10 years, we will be working on a few selected strategies that seem more promising technology analytical-wise and I really hope the focus at that point will be the standardization of procedures, for example, multicenter studies to validate the analytical and clinical performances. Now, we are still playing around with different types of probes in MS technology that can be developed for this type of application.

The bigger challenge, I think will be the acceptance of the drastic shift in the approach and the technology used for pathology. So, I'll give you the example of our brain work. In our brain work, we witness how long it took for the medical community to realize that genetic information was as effective or even more effective than classical histopathology in providing diagnostic and prognostic information. And it took a very long time to establish a consensus and understand how to use and how to interpret this information.

For example in 2007, the classification of central nervous system tumors did not report any molecular parameters in the diagnostic system, and then in 2014, the community realized that a change was necessary and then, essentially 10 years later, in 2016, the new guidelines were published

and they deemed it fundamental, the integration of molecular information with morphology and the integration in the diagnostic scheme was at the highest level.

So I think the challenge will be exactly the same when we are talking about metabolic information. The technology is right there, the technology will be available, mass spectrometry is very sensitive, it is very specific, very flexible. The problem I think will be understanding what to target, how to collect the data, and how to use it, how to interpret it for a diagnostic and prognostic purpose.

Bob Barrett: Dr. Valentina Pirro is a research scientist in the chemistry department at Purdue University. She's been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.