Article:
Daisy Unsihuay, William S Phipps, Amanda G Paulovich, Jessica R Chapman, Axel Ducret, Livia S Eberlin, Jeffrey M Spraggins, and Richard J A Goodwin.
Tissue Mass Spectrometry: How Solid Is Our Future?
Clin Chem 2023; 69(7): 676-83. https://doi.org/10.1093/clinchem/hvad061

Guests: Dr. Daisy Unsihuay is the Clinical Chemistry fellow at the Children's Hospital of Philadelphia and the Hospital of the University of Pennsylvania. Dr. Bill Phipps is an assistant professor in the Department of Laboratory Medicine and Pathology at the University of Washington.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the American Association for Clinical Chemistry. I'm Bob Barrett. Analysis of solid tissue such as surgically removed tumors or biopsy specimens has traditionally been performed by immunohistochemistry or immunofluorescence. These techniques remain an invaluable tool for clinical laboratories, providing key information that guides diagnosis and subsequent treatment, but they may have limited reproducibility, are poorly standardized across institutions, and support analysis of only one protein at a time.

With an improved understanding of complex clinical conditions that require the simultaneous evaluation of many different proteins, protein modifications, or the assessment of non-protein targets, many researchers have turned to mass spectrometry-based solutions for the evaluation of solid tissues. Mass spectrometry evaluation of solid tissues has started making inroads into the clinical space, most notably in the form of real-time testing that differentiates between healthy and cancerous tissue during surgery.

Since this initial breakthrough, other techniques with differing strengths and weaknesses have been proposed. Are these techniques ready for prime time or are there hurdles that must be overcome? What steps need to be taken to transition these techniques from the research space to routine clinical use?

A Q&A article appearing in the July 2023 issue of *Clinical Chemistry* addresses these questions and today, we're excited to talk with the article's moderators. Dr. Daisy Unsihuay is the Clinical Chemistry fellow at the Children's Hospital of Philadelphia and the Hospital of the University of Pennsylvania. Dr. Bill Phipps is an assistant professor at the Department of Laboratory Medicine and Pathology at the University of Washington where he serves as director of tissue mass spectrometry.

So Dr. Phipps let's start with you. What is the current state of solid tissue analysis in the clinical lab and why is the field ready for innovation?

Bill Phipps: Yeah. So clinical analyses involving solid tissues still primarily center on the examination of tissue morphology. This is performed by a pathologist. So this process requires staining tissues for viewing underneath the microscope. It hasn't really changed all that much for more than 100 years. What has changed, and continues to evolve, are the complementary techniques we apply to extract additional information and add granularity to this process. Probably the most prominent example in the past half-century of course would be immunohistochemistry.

So this was introduced for diagnostic purposes in the mid-1970s. It's the technique with which we label primarily proteins in tissue sections using antibody reagents. And this is really a monumental step forward in processing solid tissues, allowing for the detection of cell lineage signaling and other protein markers really both improved the objectivity involved in anatomic pathology but also facilitated the development of more personalized care, particularly in cancer. Sort of leading up to the launch of diagnostic IHC, inconsistencies in microscopy-based interpretation were pretty well documented. So there's a number of papers showing that, you know, even expert pathologists just using morphology alone, even if they're working at the same institution, would often get two different answers when looking at the same tissue slide. So I'd say it really added an additional dimension so to speak to this process and helped clarify diagnoses in many, if not most cases.

The problem is that as the use of IHC exploded and kind of continues to expand today, you know, it became subject to its own persistent challenges. So not the least of which are poor standardization and reproducibility, kind of the same problems that had been troubling anatomic pathology for so long. So, many authors have written on this issue extensively starting not too long after diagnostic IHC was first introduced. In particular, so Dr. Clive Taylor, he was a chair of pathology at the University of Southern California. He and others kind of promulgated this philosophy of what's called the total test approach to standardization.

The hope has been that with sufficient attention to detail that analysis of solid tissues could mimic the performance of testing in other clinical laboratory areas, but it's not clear even today, now decades on whether this can be achieved. IHC also struggles in other areas, for example, multiplexing, and sometimes it's used, at least ostensibly, for quantitative or semi-quantitative purposes, for which it's just not that well suited given the lack of traceable calibration measures. It

also lacks means for quantitative assay monitoring. So it may be time to kind of look towards other technologies.

Bob Barrett: Okay. Well, how could mass spectrometry address some of these challenges? Are there examples where mass spectrometry-based analysis of solid tissues has already been implemented in a clinical setting?

Bill Phipps: Yes. There are variety of configurations. Mass spectrometry, I think, I believe its positioned to close gaps and I think could serve well as a complementary technology to IHC. So in the bio fluid space, liquid chromatography tandem mass spec is often the gold standard for protein quantification.

So often, these methods are much less prone to interferences compared to their immunoassay counterparts. Specificity is also typically improved and these tests are readily multiplexed and not necessarily requiring specialized reagents in order to achieve this. Furthermore, robust approaches to calibration already exists and could likely be translated in some form to solid tissues. The many sort of possible configurations of mass spec are also a huge strength. Targeted methods, some of which have already been developed within regulated environments to help address challenges, particularly in the companion diagnostic space, where we are attempting to quantify a protein to help make a treatment decision. For example, Mandy Paulovich's group at Fred Hutchinson Cancer Center has developed multiplex quantitative assays for measuring treatment markers such as HER2 in breast cancer cells.

And then untargeted methods on a mass spectrometer allow us to detect and perform crude quantification of hundreds to thousands of proteins in a single analysis, which could greatly improve the sort of granularity with which we examine tissues in clinical environments. In terms of sort of successful clinical examples to date, while the application of mass spec to solid tissue has a very rich history in the research space, this is not really true in the clinical space. So the only sort of live clinical application to date has been what's called amyloid typing and this is still only performed at a handful of institutions across the U.S.

So, in this application, we use mass spec to identify proteins abnormally deposited in tissue sections. This is most thoroughly pioneered clinically by Ahmet Dogan and it's starting at the Mayo Clinic. The success of this endeavor has been very edifying and I think the problem of amyloid typing showcases a type of application for which mass spectrometry may be very well suited. This is because amyloidosis is a very heterogeneous group of conditions. We can identify amyloid on slides using what's called Congo red staining, but to treat these disorders properly, you have to type the underlying

protein. And unfortunately, there are at least 30 to 40 proteins and peptides that can form an amyloid deposit.

So doing comprehensive immunohistochemical investigations is just unpractical or impractical at that scale. Current assays used what's called shotgun proteomics, which consists of untargeted LC-MS analyses of trypsin-digested solubilized tissue. This allows us to identify peptide sequences, and therefore proteins, in amyloid deposits. The appeal is that you can identify all amyloid types in a single analysis, whereas, with IHC, you can apply maybe one or two antibodies per slide. The approach is also much more specific than was ever achieved using IHC. Another sort of encouraging lesson from the mass spectrometry-based typing is that it's also been an incredible learning tool. So for amyloid typing in particular, when we use untargeted analysis, it has allowed us to continually identify new amyloidogenic substances. And depending on how the data is processed, you can also identify causative sequence variants in a pathology sample.

Bob Barrett: Okay. Dr. Unsihuay, are there minimal validation requirements that should be considered when developing a method to test solid tissue?

Daisy Unsihuay: Hi, Bob. Yeah. I think the main challenge for all these methods is the reproducibility and that should be assessed at every part of the workflow, starting from the sample preparation, the analysis itself, and also the data collection. So, starting from the sample preparation, there are multiple approaches that labs can take. For instance, if it's in the case of a mass spectrometry imaging method, there's a lot of variation in the sample preparation like for instance, whether the standard can be applied on top of the tissue, under the tissue, or maybe a sandwich approach can also be taken. And we don't know how that impact into the final result.

So the assessment of these controls, it's also important and for the analysis itself, like the instrumentation, what is the reproducibility of the signals that we obtain from the instrument every day. So that's another part that is important to evaluate where a good practice this always to check on the signal-to-noise ratio, those that we get every day, or the overall performance and the detection of specific molecules that are included in the panel that we are aiming to detect.

And finally, the data analysis, we aim to apply the same approach on analyzing those results. We should follow the same algorithm so that the results are always reproducible. So yes, I think that reproducibility across different stages of the workflow is important and that would be the first approach and validation to take for this test.

And if there are not specifically guidelines for solid tissues in the CAP checklist, I started to see that there is a section in the CAP 2020 guideline for mass spectrometry imaging. That gives me a signal that the community value this technique and they are expecting that this technique will go live soon. So that checklist might be a good starting point for all those methods based on mass spectrometry imaging for instance, if they want to translate their research methods into the clinical validated methods.

Bob Barrett: Well, if these methods make the transition to the clinical setting, what kind of training and educational resources should be provided to clinicians?

Daisy Unsihuay: Okay. So mass spectrometry-based methods are categorized as high-complexity methods. So that definitely requires some training. So in case these methodologies will go live for solid tissues, we can classify those methods as the ones that will be analyzed in the lab and the other ones that are going to be used in the operating room, for instance. So the ones that are going to be analyzed the lab, we can rely that people in the lab are already trained and are qualified to make the assessment of any results that come out of those tests. But for clinicians that are actually using this technology, are starting to use these technologies in the operating room for instance, there are these new trend of those intraoperative devices that use this mass spec to analyze real-time information that can be obtained directly from the tissues.

So for that case, I think training on those positions are very important but also from people that develop these technologies, they need to make these readouts very simple, so we don't scare them to use this technology. So there's a basic knowledge of biomarkers that is important for clinicians to know, but if those algorithms are very complicated, so then they won't be usable by clinicians. So, I think that's important to make those technologies more intuitive, easy to interpret, so that we can see more of these devices coming out to the market and being applied, and in the operating room, which is the end goal for this devices.

Bob Barrett: Well, finally are there any other techniques that could be coupled with mass spectrometry to analyze solid tissues and improve diagnostic capabilities?

Daisy Unsihuay: Yes. The one that I'm seeing that is becoming very promising is ion mobility. Ion mobility is a technique that can separate molecules based on their mobility as their name indicates. And that is related to the mass, charge, and shape of the molecule, and they are flying in a chamber full of gas.

So the interesting part of this technique is that they operate in the millisecond time scale so they can do this task of separating molecules much faster than the liquid chromatography platform. And this technique has found a lot of applications in research setting because it can help us to separate molecules, improve their resolution, and also detect for isomers. And if we think about nature, there are a lot of biomolecules that have isomers and most of them are also relevant and can also be potential biomarkers.

So that is the interest where ion mobility might find an application in the clinical setting. Aside of all of it, it can help measuring what we call a collision cross section, which is a molecular descriptor that also adds another level of confidence in identification of molecules. So why is this important? Because when we don't have enough sample for instance, and we can just measure the mass of something, but we cannot fragment to confirm the identity. But if we can measure the collision cross section, that can also help us to identify a molecule.

So in untargeted metabolomic approaches, so this is very useful because in that type of experiments, we don't have the luxury of running fragmentation, when we do this untargeted approach. So by using those collision cross section measurements, we can definitely identify, add another extra level of confidence in the molecules that we claim to be. But to translate this into the clinical setting, clinical setting mainly relies on targeted approaches where specific molecules are measured and quantified. And I think this is a main limitation for this technology because so far I haven't seen a lot of quantification approaches for ion mobility and I think it might be related to the decreased sensitivity that happens when we couple this technology to mass spectrometry.

So I think in the coming years, different companies are tackling down this problem so that sensitivity is not compromised and that we can measure confidently those amounts of analytes accurately in tissue. So I think in the coming years, once we tackle down this limitation, we will see more of these applications and finally, it might be a huge advantage to implement it in the clinical setting because it operates much faster than liquid chromatography platform. So all of them can work together to improve the overall workflow of the clinical test.

Bob Barrett: That was Dr. Daisy Unsihuay from the Children's Hospital of Philadelphia and Dr. Bill Phipps from the University of Washington. They served as moderators for a Q&A article on tissue mass spectrometry in the July 2023 issue of *Clinical Chemistry*. And they've been our guests in this podcast on that topic. I'm Bob Barrett. Thanks for listening.