Contemporary Challenges in Clinical Flow Cytometry: Small Samples, Big Data, Little Time

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Guest: Dr. Jon Brestoff is an Assistant Professor of Pathology and Immunology at Washington University School of Medicine and a Medical Director of the Clinical Flow Cytometry Laboratory at Barnes-Jewish Hospital in St. Louis.

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. Flow cytometry is an analytical technique commonly used by clinical laboratories to characterize the amount and phenotypic features of immune cells or neoplastic cell populations in samples derived from patients. The information gathered from flow cytometry analysis can aid in disease diagnosis, prognosis, and the monitoring of residual disease. However, clinical flow cytometry laboratories often face several challenges such as suboptimal specimen quality, efficiency of laboratory workflow, and increasingly complex datasets. Improvements in analytical technology data analysis and storage and result reporting are necessary to optimize the quality of these tests for patient care.

In a review article published in the July 2022 issue of *JALM*, the authors summarized the state of clinical flow cytometry testing today and the major challenges currently experienced in this area while providing proposed solutions and strategies for these challenges. The authors also review emerging technological improvements in cell analysis. Today, we're joined by the first author of the article, Dr. Jon Brestoff. Dr. Brestoff is an Assistant Professor of Pathology and Immunology at Washington University School of Medicine, where he leads a research laboratory studying the immune system. He also serves as a Medical Director of the Clinical Flow Cytometry Laboratory at Barnes-Jewish Hospital in St. Louis. Dr. Brestoff, welcome.

Jon Brestoff: Thank you so much for having me.

Randye Kaye: Let's start with the basics. What exactly is flow cytometry and what is it used for?

Jon Brestoff: So, flow cytometry is a way to identify different cell types and blood, bone marrow, and other body fluids, and we use it in a clinical setting, usually to diagnose and monitor various types of cancers, some different blood disorders, and immunodeficiencies. And we also use flow cytometry to count

stem cells in preparation for bone marrow transplant and some labs also use flow cytometry to support quality control for cell products that are made, like CAR T-cells.

Randye Kaye: Wow! So, that sounds like a really important piece of technology. Can you explain a little bit about how it works?

Jon Brestoff: Sure. So, cells are stained with many different types of antibodies that are attached to different colored dyes called fluorophores. The cells are then passed in a single file line in front of one or more different lasers that excite the fluorophores. And when the fluorophores relax, they emit light in certain wavelengths and that light is detected. So, this allows us to see whether cells are stained, for example, red and blue, or red and green, or blue and yellow, etcetera, and using the different patterns of color combinations on each cell type, we can define what those cells are, and pack together a bunch of different antibodies into a single different test so that we can identify various cell types and whether or not those cells look normal or abnormal. So generally, the more colors that we can fit into a test, the better, because it allows us to get more information about a patient.

Randye Kaye: Wow! So, can you tell me a little bit more about some of the new and the emerging technologies in this field?

Jon Brestoff: The technology that excites me the most right now in flow cytometry is called spectral flow cytometry. So basically, the way that spectral flow cytometers work is that they can see many different shades of every color in the visible and ultraviolet light spectra. So, when each fluorophore emits light, typically there in a normal flow cytometer it's just measured in a specific wavelength, just one wavelength. But spectral flow cytometers can see the entire unique pattern of emitted light, much like a fingerprint. So, using this technology, you can pool together well over 40 different markers into a single test. I think a really good analogy for this is like looking at the wall of paint chips at home improvement stores.

So, your eyes can easily tell the difference between all the different shades of red, blue, green, purple, and spectral flow cytometers can do the same kind of thing. But conventional flow cytometers that are used right now can only see this predefined sliver of light. In the paint chip analogy, that's the equivalent of just being able to see light blue, medium blue, and dark blue, but not the 15 different shades of blue that might be represented on the wall. So, spectral flow cytometers just allow us to see so many more colors and to build much better tests that allow us to do a lot less work with one sample.

Randye Kaye: Wow! And that's a really terrific analogy. Thank you for that. So, you have the ability with this to measure so many all at once. What are the benefits to the clinical laboratories and the benefits to patients?

Jon Brestoff: That's a great question. So, one of the biggest challenges is that we often deal with small specimens. A lot of the samples that we work with come from babies or kids, where it's really difficult to get enough volume of blood from these individuals. Or the samples may come from patients with cancer, or they may come from cerebrospinal fluid, which is, you know, got very low cell counts to begin with. And so, the cellular content of these specimens can be very low. And the problem with that is that sometimes we have to split up each sample to be able to perform a single test.

So, a sample might be split two, three, or four different ways to provide the ordered tests, but we can't do that for some of these patients. And so, what ends up happening is some of those tests just get canceled because of insufficient quantity of cells. So, spectral flow cytometry would allow us to combine these assays into as few tubes as possible so that the rate of insufficient quantity of cell cancellations goes way down. Ultimately, that also leads to a lot less work for the lab and it is obviously better for patients. So really, in my view, it's a win-win.

Randye Kaye: And absolutely sounds like a win-win. So, are spectral flow cytometers available now for clinical use?

Jon Brestoff: They are available for clinical use in China and Europe. But to the best of my knowledge in the United States, it's currently only used in a research use only setting. Actually, my research lab has a four-laser spectral flow cytometer and it's amazing. We can build flow cytometry tests in a research lab that have basically outperformed every other flow cytometer that I've worked with or encountered in 14 years of science and medicine. But this technology is not yet available for clinical use in the United States. And in order for that to happen, a spectral flow cytometry manufacturer would need to obtain regulatory approval for their device with the FDA. And personally, I really hope that that happens soon because I think it's going to make a huge difference for patients and labs alike.

Randye Kaye: Well, you've convinced me. I certainly hope that happens soon as well. Finally, can you just leave us with maybe some closing thoughts or key takeaways on the future state of flow cytometry in the clinical lab?

Jon Brestoff: Well, one of the challenges I think the flow cytometry labs face is that the technology itself is very expensive, and it's the field has faced really difficult challenges to the

reimbursement schedules for flow cytometry, which makes it financially challenging to operate. But it's such a high value technology for patient care. We simply can't let it get to the point where hospitals and labs decide it's not worth it or financially viable to offer in-house flow cytometry testing, leading all those tests to be sent out to reference labs. Ultimately, that's not good for turnaround times. And when you're sick and need to know the answer to flow cytometry tests for your care, waiting a couple of extra days can have a huge impact. So, because of that, I think we really need to rethink how flow cytometry testing is organized and how it's performed in order to keep that part of the lab at the forefront of innovation.

Randye Kaye: Sounds definitely worth it. Thank you so much for joining us today, Dr. Brestoff.

Jon Brestoff: Thank you so much for having me.

Randye Kaye: That was Dr. Jon Brestoff from the Washington University School of Medicine describing the *JALM* review article "Contemporary Challenges in Clinical Flow Cytometry: Small Samples, Big Data, Little Time." Thanks for tuning in to this episode of JALM Talk. See you next time and don't forget to submit something for us to talk.