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Toward Clinical Application of Leukocyte Counts Based on Targeted DNA Methylation Analysis

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Guest: Dr. Wolfgang Wagner from the Institute for Stem Cell Biology at Rheinisch-Westfälische Technische Hochschule, Aachen University Medical School in Germany.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Analysis of the types of white blood cells is among the most frequently requested tests in the hematology laboratory. Leukocyte differential counts can be determined by microscopic evaluation and manual counting, as well as by automated cell counters. But it has recently been shown that leukocyte counts can also be determined by cell-type specific DNA methylation. Such epigenetic leukocyte counting is applicable to very small blood volumes and even frozen material.

A paper appearing in the May 2022 issue of *Clinical Chemistry* has further examined clinical application of leukocyte counts, based on targeted DNA methylation analysis.

The senior author of that study is Dr. Wolfgang Wagner. He is Director of The Institute for Stem Cell Biology at Aachen University Medical School in Germany, and he's our guest for this podcast.

Dr. Wagner, leukocyte counts are one of the most frequently performed clinical tests, so why do we need another method in addition to cell counters in manual microscopic identification?

Wolfgang Wagner: Yeah, that's a good question because of course there are well-established assays like, manual cell counts, automated cell counters, flow cytometry, and there's always a little bit of uphill battle if you want to make a new system for a clinical test, but the conventional methods too have shortcomings. You always need to have fresh blood. So, it doesn't work with frozen blood or it cannot respectively analyze samples that they have harvested weeks or a month ago. And it doesn't work for all samples because sometimes blood samples coagulate, for example, or for some reasons you cannot

analyze them and then it may be good to have an alternative approach.

Anyway, cross-pollination between different methods is always important, also for conventional and well-established assays, and, for example, for flow cytometry you need a larger volume. So that wouldn't work for small blood traces like capillary blood, for example. And last, but not least, even with the well-established assays, standardization is an issue. We saw that when we compared data in a large ring trial and it was surprising how much variation there is between the established cell counters. So, I think there's room to improve.

Bob Barrett: How does Epi-Blood-Count that you describe in your *Clinical Chemistry* article work and could you tell us a bit more about epigenetics in general?

Wolfgang Wagner: Epigenetics is really what drives cellular differentiation. All of our cells have this very same DNA and what really makes the cell different into a skin cell or heart cell, or a neuron, is actually epigenetics. These modifications are on the DNA strand that tell which genes can be activated and which are inactivated. So that really is what drives cellular differentiation. It's therefore ideal to characterize the different subtypes.

In fact, there are different flavors of epigenetics and here we are particularly focusing on DNA methylation that is methyl groups that are added to specific cytosines on the DNA strand and these methyl groups can be measured on a single-nucleotide resolution at a relatively high precision. So that's why we believe that this is actually ideal as a biomarker to specify the individual cell types.

And as we know, our blood has many different leukocyte subsets, for example, different lymphocytes such as B cells or CD4, or CD8-positive T cells, NK cells or granulocytes, all different kinds of granulocytes and monocytes. So, the specification of all these leukocyte subsets, we can do quite reliably by these DNA methylation patterns on the DNA.

So what we need to do are the assays we need to isolate the genomic DNA. It's quite simple or having said that of course this doesn't work for red blood cells or erythrocytes and also not for platelets, for thrombocytes because these cell types don't have DNA and therefore we cannot analyze them with our Epi-Blood-Count approach.

But for the other cell types, we can just simply isolate the genomic DNA and then we are analyzing DNA methylation, very specific regions in the genome, and that's actually something that we developed in our assay that we try to

pinpoint those sites on the genome that have very specific DNA methylation patterns. And with these targeted assays we can then, for example, with pyrosequencing or digital droplet PCR determine the DNA methylation and use this for deconvolution to estimate a cellular composition in the blood. We did this, of course, in comparison to conventional cell counts and then the precision was fairly good.

Bob Barrett: Doctor, is there a gold standard available for leukocyte counts and have you used it as a comparison method in your studies?

Wolfgang Wagner: Well, leukocyte counts are used in many, many different applications, in many different backgrounds, so there is not the one gold standard, there's different methods that are used, and also for the different cell types can be combined in different ways.

But of course, we did compare this in comparison to these conventional or if you want to say gold standard cell counts. So, for example, they have automated cell counters that are used in our departments for hematology or transfusion medicine. These automated cell counters are basically working on the size and granularity of different cells. They can specify the cell types quite good, but not the individual lymphocyte subsets.

If you want to discern, for example, B cells or different T cell subsets, what you need to do is you need to label these cells with antibodies for flow cytometry. And as I said before, this needs a little more cells, it's also a very labor-intensive and difficult to standardize procedure, but that's something that is of course also gold standard in the clinics and that's also something that we did together with our department of immunology so they analyzed the samples, the same samples with the flow cytometry measurements.

And let's say, a gold standard in general to analyze leukocytes subsets is really still the manual counting. It's very labor-intensive, but still, you can do this also, it's also automated, but with Machine Learning approaches and microscopy, but still does as technicians working in the lab that are inspecting the blood smears to determine the number of different cell types in the blood, particularly if you have a blood sample that's not so easy and then it seems to have some aberrations.

And again, this is something that we compared with our Epi-Blood-Count approaches, and overall, the correlation was not so bad. So, I think, at least we are on a good way to establish a method based on DNA methylation that can compete with so-called gold standard methods.

Bob Barrett: Blood counts from small finger-pricks may open opportunities for self-testing. Could you tell us how your Epi-Blood-Count can be applied in various clinical settings?

Wolfgang Wagner: That's a good question too because, of course, we all know how blood is usually collected. It's not a very pleasant experience and of course, we need to have a certain volume of blood here. It would be much easier if we could simply use a small finger-prick. So, to just collect a drop of blood, particularly if you consider, for example, elderly people that cannot go to the practitioner so easily or as we all experience now with COVID, people in quarantine. How could they analyze their blood if they don't want to go to the doctor? And also, I think there is an increasing percentage of people who want to do self-tests to survey their health themselves that perhaps don't want to go to a clinician for conventional blood collection.

So, I think finger-pricks would really make life and diagnostics a lot easier. Also, since conventional blood drawing is not always easy, there's many patients where their veins are very small and fragile and collecting blood is really difficult. So, what we can use with our Epi-Blood-Count is that we really only use a very, very small blood volume that can be collected with a sponge or with a paper tissue. So, let's say, 30 microliters would probably be more than enough. And this blood can be dried and shipped at room temperature, you can ship it for more than a week without a problem and then this can be analyzed at a specified lab for our Epi-Blood-Count approach.

So, this may be quite attractive, as I said, for certain applications. We still need to better implement here the absolute quantification, which is still a little bit difficult, but that's something that we are also working on. So, I think that in the future, maybe at least for some of the blood tests, it will not be necessary to collect blood from a venous puncture, but already from a finger-prick.

Bob Barrett: Finally, doctor, in your opinion, do you believe that the method is sufficiently validated and ready to be applied in routine clinical laboratories; and if not, what steps still need to be taken before it's ready for primetime?

Wolfgang Wagner: Yeah, that's a good question again. So, now we're not there yet. I think what we have shown is a proof of concept that the method could be translated to the clinic. We have so far tested about 400 healthy donors and 300 disease patients. And overall, I think the predictions matched quite good to our expectations, but not for all cell types. There are many cell types where we need to optimize our assays.

I think in general the translation of our system is much easier to other approaches. Epigenetics are used for cell type specification because what we're doing here is only looking at very, very specific cells in the genome, so, there's no need for genome-wide assays. This is not dependent on a specific microarray platform, for example. It doesn't need sophisticated bioinformatics.

So, I think in general, the method can be applied to the clinic, but there's still a long road to be taken. For example, I think there's a much better qualification needed.

We, for example, could demonstrate that if you have a leukemia, which of course entails epigenetic aberrations too, that this also affects our epigenetic blood predictions. Then there are scores that can be used to discern but I think in this case the analysis should be complimented with other means or with other conventional methods, of course. And there is also many requirements because in the end, our assay would need to be certified as an in vitro diagnostics device, which has very harsh regulations in all kinds of countries. And then, of course, this goes with a better documentation of the procedures and also better testing. The whole procedure also probably should be automated to really bring it broadly into the clinic. So there needs to also be our coding system for handling of the different specimen.

So, yes, I think in principle, the method can be translated into a clinic and I think we are on a good track, but still there are many things that we need to optimize and improve.

And of course, last but not least, to really have these methods available for the patients, also the clinicians need to be informed about these new approaches and understand a bit of the background. That's why I hope that also, this podcast here can help to bring a better awareness of the possibilities that are now arising with Epi-Blood-Count.

Bob Barrett: That was Dr. Wolfgang Wagner from the Aachen University Medical School in Germany. He has been our guest in this podcast on Clinical Application of Leukocyte Counts Based on Targeted DNA Methylation Analysis. He is an author of a paper describing that approach that appears in the May 2022 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.