

Host: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

Newborn screening programs, mandated by law in most jurisdictions, are aimed at the identification of children with clinically treatable disorders.

Delays in the diagnosis of some of these disorders can lead to permanent physical or mental impairment or even the death of affected children.

Screening methods employed for some disorders rely on detecting high blood concentrations of biomarkers that are not intrinsically diagnostic for the underlying conditions being screened. In such cases, the question of how to define appropriate biomarker screening concentration thresholds that avoid a high rate of false-positive screens, while maintaining an adequate rate of detection of the disorders, can be difficult to address.

This problem holds true for the detection of inborn errors of propionate, methionine, and cobalamin metabolism, where the screening relies on finding abnormally high concentrations of methionine and propionylcarnitine.

However, because abnormally high concentrations of methionine and propionylcarnitine are not specific for detection of these inborn metabolic defects, many false-positive screening results are currently generated.

An article published in the November issue of *Clinical Chemistry* reported on the development of a method to improve this situation by use of liquid chromatography–tandem mass spectrometry to detect total homocysteine methylmalonic acid and methylcitric acid in dried bloodspots.

Dr. Dietrich Matern is co-author of the report and Associate Professor in the Departments of Laboratory Medicine and Pathology, Medical Genetics, and Pediatrics at the Mayo Clinic. He is our guest in this podcast.

Tell us, Dr. Matern, what exactly is newborn screening?

Dr. Dietrich Matern: Newborn screening is a public health program that aims to identify conditions, where early intervention can basically prevent mortality, morbidity, and disabilities. And this has been around since the early 1960s, and was introduced by Dr. Robert Guthrie, who became involved because he had a son with mental retardation, and although the cause of that was never found out, he got involved with a lot of pediatricians, neurologists, with parent support groups.

And in the 1950s, heard about the condition, phenylketonuria, which is inborn error of amino acid metabolism, where phenylalanine cannot be metabolized to tyrosine, and that was identified to cause mental retardation.

And in the early 1950s, treatment became available by basically reducing the phenylalanine intake in the diet, and that showed to help patients, although it was not a cure, and that was because patients, once they develop symptoms, which is usually after they are six months old and the physician knows that they don't meet milestones, they may have seizures, you cannot go back, the damage is done, and it will not normalize.

So what Bob Guthrie then was wondering about, that may be if we identified these children before they developed symptoms, because again, they were born clinically fine, had no apparent problems, and if you introduced the diet earlier, then maybe you could prevent all the negative sequelae.

Then he thought, well, how could we do this, and he was working on bacterial inhibition assays to measure cancer drugs in blood, and then he started screening serum samples for phenylalanine in affected patients, but again, when you thought about finding these patients early, he thought up the idea of doing bloodspot collection before the baby leaves the hospital, collecting blood after a heel stick, on a filter paper, drawing that, and then sending it to a centralized laboratory, that measures the phenylalanine level in these samples.

So he did that first locally in Upstate New York, where he was living, and then basically it took the world from there, and now all developed countries at least screen for phenylketonuria and many other conditions.

And many other conditions really became an issue in the late 1990s when tandem mass spectrometry was applied to newborn screening. And that allows that you do not measure only a single analyte, such as phenylalanine in a bloodspot, but it allowed you to measure multiple amino acids and acylcarnitines in these dried bloodspots, and basically would provide information whether that patient is affected with one of more than 40 different inborn errors of metabolism.

So that is, again, done on bloodspots. More recently now there is also a newborn screening that includes hearing loss, and in the near future probably congenital cyanotic heart disease, both of which are tested on, on the bedside and not on bloodspots, but that is newborn screening.

Host: With that in mind, what are second-tier tests?

Dr. Dietrich Matern: Second-tier tests are a way to reduce the false-positive rate. Now, the screening is done for biomarkers. We do not screen for a specific disease. We screen for markers that are indicative of the presence of disease.

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And some of the biomarkers, there is significant overlap between the concentration range for the marker in the normal population and in affected patients. So if you want to catch all the patients, you have to lower your threshold for that marker, where you however will have a higher false-positive rate. If you want to avoid the false-positive, you have to increase your cutoff and then you will miss some of the patients.

So the second-tier test can help here, because what can be done is you can look using a different test, looking for more disease-specific markers.

So the first test for this—or condition for which this was done was cystic fibrosis in the 1990s, where immunoreactive trypsinogen is the first-tier test, and when that is abnormal, which happens in a lot of babies that are not affected with cystic fibrosis, then they did or are doing now a second-tier test for specific CF mutations, so that helps a lot.

What we have done here at the Mayo Clinic is we have developed few biochemical assays based on tandem mass spectrometry, looking at specific—disease-specific markers for some of the analytes picked up by newborn screening, and apparently, most recently looking for methylmalonic acid, methylcitric acid, and total homocysteine in samples where the primary newborn screening test reveals an abnormal propionylcarnitine or methionine.

And again, both of these markers can be relatively high in unaffected newborns, that could be for methionine, because the patient is receiving parenteral nutrition or other reason that we don't yet understand.

But it could also be mildly elevated in truly affected patients, so that's where the second-tier test is very valuable to figure out, what is going on in the particular baby without having to contact the baby's family.

So basically the way we are doing the second-tier test when we have an abnormal first-tier result, we do not ask for a repeat specimen. We actually take the existing specimen, take another bloodspot punch from that sample, and do the specific analysis. And again, in more than 90% of the cases, the second-tier test is normal, which overrules the abnormal first-tier result, and so the family and the physician caring for the baby are never bothered about the first-tier result.

Host: So what are the advantages? What's good about these types of tests?

Dr. Dietrich Matern: Well, in addition to what I said, the benefit is several fold, because you don't have to create unnecessary anxiety, and you don't also have to invest in expensive follow-up testing of the result, that in most cases turns out to be false-positive.

So there will be no additional clinic visit for the family, and the physician will not have to take the time and the family doesn't have to come back for that visit, and there will be no superfluous laboratory testing.

On the other hand, if the first and the second-tier test are both positive, then the follow-up physician can be more assured that the baby is very likely affected and can counsel, treat, and follow up accordingly and without delay.

Host: In your opinion, are these second-tier tests really necessary?

Dr. Dietrich Matern: In my opinion, yes, because I believe that most people rather deal with two results than false-positive results. As mentioned before, the conditions we screen for in the newborn period are potentially lethal. So you can imagine what happens when a family is informed about such a possibility. They will be very worried. They will go home, turn on their computer, look online for more answers, and are likely to find descriptions of the worst-case scenarios.

So if there is a cost effective way to avoid putting a patient and their family through significant worries, which actually have been shown to sometimes have detrimental effects on the parent-child relationships, yes, then I think we should pursue such improvements.

Also, the number of conditions included in newborn screening programs continues to increase, and will include, in the very near future, several Lysosomal Storage Disorders.

Actually in New York, already screening is being done for Krabbe disease since 2006, and now in Illinois, their program is about to start to screen for five Lysosomal Storage Disorders, and then Missouri and New Mexico will follow in a few years.

So with every condition you will add, you will increase the overall false-positive rate, which again will increase anxiety and cost for unnecessary follow up. So when it comes to cost, I could give you an example.

In June 2004, we implemented a second-tier test for congenital adrenal hyperplasia screening and applied it here to the newborn screening program in Minnesota. What this allowed us to do is reduce the false-positive rate from nearly 1% to less than 0.1%. And if you were to try to calculate the cost and the follow up, and for the second-tier test, before and after implementation of the second-tier test, we actually now save about \$3 million since 2004 in the follow-up cost.

So this is money that is not showing up anywhere, but it is not spent anymore.

Host: Can NBS Laboratories perform these types of tests?

Dr. Dietrich Matern: I think Newborn Screening Laboratories can perform these tests, and some are doing this already. So the assay for congenital adrenal hyperplasia, the second-tier assay is in place, in several laboratories in the United States and other countries, and we typically have no qualms in providing our SOP to interested screening laboratories.

We have brought up several second-tier assays over the years, and again, laboratories are looking at those, sometimes they come up with their own solutions, but yes, second-tier test can be performed in Newborn Screening Laboratories.

We use, as in the most recent case, tandem mass spectrometry, which is again, already present in all newborn screening labs, so if they utilize their equipment the right way, then I think they can do that.

Host: Well, you say these tests do eventually save money, but they cost money too. Do Newborn Screening Laboratories have budget set up to take the test and run them?

Dr. Dietrich Matern: That probably depends on the program. However, I think what most newborn screening programs and states have, again, these are state mandated, and usually every state has an Advisory Committee on newborn screening, and I think if you can provide information, and it is out there now, what kind of savings you can achieve by doing the second-tier test, it should be really a no-brainer.

Also, you do not necessarily have to run all these tests in your laboratory. You can work together with other screening laboratories, so that one screening lab has some capacity on their equipment to run one second-tier test and another screening lab has the capacity to run another one, and if they work together, they can basically send their samples to the other screening lab just to do the second-tier test.

And we do this year, we have a few labs that do send us testing, particularly for congenital adrenal hyperplasia, but now also for the new assay for methylmalonic acid, and methylcitric and total homocysteine as a second-tier test. So I think that it is possible to regionalize the second-tier testing so that everybody can benefit.

Host: Dr. Dietrich Matern is an Associate Professor of Laboratory Medicine and Director of the Biochemical Genetics Laboratory at the Mayo Clinic. He has been our guest in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening.

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