

Severe Combined Immunodeficiency National Monthly Conference Call and Webinar

July 2016

Presentation: Abnormal TREC in Premature Infants and Postnatally

Speaker: Lisa Kobrynski, MD, MP

Please direct all comments/questions pertaining to this presentation to Ruthanne Salsbury at Ruthanne.salsbury@aphl.org or 240-485-2767

Ruthanne Salsbury: Okay, great. Good afternoon everyone. This is Ruthanne Salsbury on the line and on behalf of new steps and the newborn screening translational research network I'd like to welcome everyone to this month's SCID national webinar. We're so glad that you all are able to join us and for those of you that might be new to these calls... These calls address legislative and regulatory challenges, laboratory methodologies, and techniques and follow up activities relating to SCID.

Before we get started I just wanted to share some reminders with you. All of the lines have been muted, but please feel free to write questions in the bottom left corner of the screen. I will also be opening up the lines at the end of this call, so please feel free to ask questions through your phone line at that point.

Today we're going to begin with the agenda, so we'll begin with an updated map of SCID status across the United States and then we are so delighted to have Dr. Kobrynski present on abnormal tract and premature infants post-natally and prematurely. Then we have allowed for a lot of time for questions and discussion, so please save your questions until the end. We're really excited to have Dr. Lisa Kobrynski here to answer some of your questions.

Lets begin with an updated map of SCID newborn screening. Since we last spoke in June we are so excited to announce that two more states are now screening for SCID and that's both North Dakota and Vermont. We are so excited that they've begun screening and I encourage you to check out our website at www.newsteps.org/scid to continue to get an updated status for each of these states. I just received notice that Alaska also started screening on July 1st, so hooray that's great! Thank you so much for sharing that.

I'd now like to hand it over to Dr. Lisa Kobrynski. If you wouldn't mind just pressing star seven and we can move forward with your presentation.

Speaker 2: Hey, thank you, Ruthanne.

Ruthanne
Salsbury: Thank you.

Lisa
Kobrynski: I'm going to talk actually a little bit about cause you know, Georgia is late to the game started screening. I'm going to speak a little bit about what we've been seeing and a couple trends that are maybe a little worrisome. I don't know and some other states can chime in on their experiences, especially ones that have had bit more experience. Some of this was initiated after we heard from Maryland how their second screen in seven days they were getting back a lot of TREC values that were outside their cut off. I gave my disclosures to Ruthanne, but I do do some clinical research for Grifols and Baxter and I work at Emory University in Children's Healthcare of Atlanta. Next slide please.

Georgia, just to put out some [contracts 00:03:22], so you have about 135,000 births a year. About 10.8% are less than 2,500 grams, so we have a fairly high rate of premature births and the table shows you in comparison to a lot of other states where we fit. We rank, we get a grade of F for [inaudible 00:03:42] the public health divisions for our pre-maturity. We do have a fair number of premature births and much worse obviously in the urban areas like Atlanta and Augusta. Next slide.

I was trying to look back at other states and get some idea of how many of their premature babies are showing up with abnormal screens and then how many are actually referred for flow cytometry. Among... In the JAMA study, which is a study that came out a couple years ago where they looked at several states... I've picked those states that have been actually screening for a fair amount of time and had a good number of births.

One can see that out of this fairly significant number of births you have over a million in California, [inaudible 00:04:33] 71,000 in Colorado. The number of babies referred to flow was fairly significant I mean 206 in California, 108 in Wisconsin... The number of preemie infants that were referred for flow was actually fairly small. I think some of this is because what they choose as their critical value. In other words, my understanding from most these states is that they will only do flow on a premature baby if the TREC is above or below their critical values, in other words they have no detective TREC or it's extremely low. That gives them a fairly low rate of babies that are referred for flow. Next slide.

New York is one of the few states that's published data about where the TREC numbers tend to lie based on gestational age. Now I know that many states don't report gestational age because it can be rather inaccurate and Georgia is going to start reporting gestational age soon. Right now we only report birth rate and someone can... Someone could assume that a baby that is an extremely low birth weight, so somebody's that's less than 999 grams is gonna be a baby that's probably going to be less than 28-29 weeks of gestation.

From this paper that was published by New York in 2014, one can see that the median overlaps very well between the very premature babies and the term babies, however,

when you look at the number of babies that fall well below that, it does tend to be quite a bit higher than the term babies. Of course the number of TRECs can increase quite [markedly 00:06:27] when you're at term. What this means is that, at least in this paper, they published that for their term babies a critical value was if they had less than a hundred and 25 copies of TREC per microliter of blood compared to a premature baby that had less than 200 copies of TREC per microliter of blood. Now most states I don't think have incorporated this into their algorithms. Next slide.

This is another graph from Connecticut that looked at the differences in their TQ. This is in [zero 00:07:05] point copies of TREC, which they also do based on the calibrator. Their actually giving you the threshold based on the... The reading that you get from your PCR machine of the TREC. The green line is the front line is the normal birth rate and the red line is the extremely low birth rate babies, which are those less than 1000 grams. One can see that where the dotted line falls is what they consider a cut off for really undetectable TREC and this is only... This is going to get you well less than 1% of your term babies, but nearly 20, well a little more than 20. About 22% of your extremely low birth rate babies, so a very, very significant percentage. Next slide please.

In Georgia, we fall [inaudible 00:08:07] unlike most states is that if we have a baby at any gestational age whose TREC is abnormal and under a critical value we refer them for flow. In a premature baby, so in our case in a baby less than 2,500 grams, if the TREC is abnormal, but not critically low we will repeat the newborn screening test and that repeat often happens frequently because of other metabolic diseases. Ideally we would probably not do it for at least 2-four weeks, but in practice it often gets done more frequently than that because of other metabolic issues.

When I... When I looked at our... I asked our state lab to kind of give us an idea of what we're seeing of the babies we have that come back with abnormal TRECs and remember that our abnormal is considered CQ of over 35.5 and a critical is a CQ of over 38.5. The mean TREC for the babies that were 0-999 grams was 37.4 if they were little less than 1,500. The mean was 35.45, but there was a spread with the median being 37 and if they were 1,500-2,499 the mean was 36.9 and the median was 36.5. Now this is not all of them, this is just the ones that turned out to be abnormal. Next slide.

Looking at this in terms of percentage, you know, you want to not have too many of your percentage falling in your critical range so you can compare the babies that are over 2,500 grams about 0.1% of them were referred for flow cytometry. Of... These were because they had abnormal TREC and of these, 0.2% were critical abnormal. For the... Compare this to the babies that are 0-999 grams where 11.5% of them would be considered abnormal and would normally be referred for flow, but of these, eight of them were critical abnormal. Very significant proportion of these premature babies not only were abnormal, but they had critically abnormal results. It wasn't very high for the 1,000-1,499, but this is based only on a small sample. It's only 12,000 births, so it's not a lot. Then the intermediate group, it was not so bad with only two of them being referred because they were critically abnormal. Next slide.

What happened to these very premature babies who are not... Well they aren't all very premature, but what happened to the ones that had these abnormal TRECs. This just shows you some of the ones and the kind of CQs that we got. They varied between 38.9 and 43. Remember, a value greater than 38.5 is considered critical, so in all these cases they were asked to have flow. Although the ones where it says a repeat was normal, this often happened because of a metabolic issue that they had repeated it in the [interum 00:11:38] and the TREC came back in a normal value. The first baby that was 500 grams, that's what happened. There were several of them where the flow is still pending on these babies probably because it's difficult to collect any extra blood from these very small babies who are quite sick in order to get flow cytometry. We had another baby who was extremely premature whose only 400 and something grams who really did not survive long enough to have flow done. Next slide.

One of the things that we need to decide, or at least we're looking at in our state, is to decide with these very low birth weight babies, how do we look at the critical values. Should we consider like they were put in, at least the paper in New York, that we should be looking at a separate cut off for criticals or should we look at them by case by case basis? When we look at... When we talk to the NICUs for these premature babies, nearly all of these are suffering from complications of prematurity. They can have kidney failure, they can have problems with bowels, they may have a lot of problems with just fluid losses, and nearly all of them are getting transfused with leukodepleted irradiated blood.

If not daily, every other day, so in practice this isn't a large amount of volume of blood being given at a single time, but over a couple weeks of time you may have a baby that's gotten five or six transfusions. That's in addition to total parenteral nutrition and other types of fluid that they're getting, which we don't know at this time. It might be that this could interfere with our measurements of the T cell numbers and the lymphocytes. We do know that at least one of the babies where it was, had flow. The baby was, looked very abnormal and part of this was because there was pancytopenia. Meaning all the lages were suppressed. White count was low, the hemoglobin was low, the platelets were low, and the baby was extremely, extremely sick. The other one unfortunately the flow was sent, but the correct test was not sent, and so it couldn't be interpreted.

The other issue that is a problem is that most of the NICUs now start trophic feeds, which means they start a very small amount of feeding to the premature baby through a nasal gastric tube with breast milk and if the mom is pumping, maybe mom's breast milk otherwise it'll come from a breast milk bank. We did have one baby early on who had CHARGE syndrome and was a complete aplasia of the thymus winded up with CMV, which may have been through or transmitted through breast milk. I know that bank milk is pasteurized, but the maternal breast milk is generally not. This is another issue that we're dealing with. I'll just finish up and then I guess we can open up to some questions. Next slide.

We want to try to see if we can gather a little more information especially for these premature and very low birth weight babies. What is the population mean, median look like for the entire population? Are we seeing a frequency of abnormal TREC or really critical values that are well outside what we see for the more term or higher birth weight babies. Does this mean that we should be looking at different cut offs for these extremely low birth weight babies in terms of determining whether or not it is a critical value? That's really the only value that affects our decision making because the lows, but the non-criticals generally just results in us repeating the specimen and not actually trying to send flow cytometry on this very premature baby, which is then very difficult to accomplish. Next slide.

The other issue I want to raise was also... Was really actually started with a discussion with Maryland was where they were seeing babies that were screened at birth and then some days later. Their TREC value was decreased at seven days, which sometimes led to the second [inaudible 00:16:32] specimen being read as abnormal. We... I've talked to a couple other states that do two tests and they've not reported seeing that.

I know that one other state has not seen that either, which is Texas. I don't know how big a problem that is, but what we've seen in Georgia was we've had now four babies who were really essentially normal. They are not premature, they are term babies. The older babies, the nine month and the ten month old babies actually were well thriving, perfectly fine. This newborn screen was done at a much later date and we did... At least in the case of the third baby where it was done at nine months of age, we were lucky enough to be able to retrieve this [thought 00:17:23] to know that it was normal at birth. The others, we never... We could not retrieve the results [bot 00:17:30] or it wasn't sent. For those babies we still have a difficulty in knowing what does this mean? What is normal? What is not normal? Next slide.

We know that when babies are born they're generally... Their total white count is higher and their absolute lymphocyte count is much higher at birth. We know that it drops during the first year of life. Not horribly, so in other words if a normal term baby maybe has 6,000 lymphocytes per cubic millimeter, by a year of age that might've dropped to 5,000, which should not overall make your TREC look abnormal even with our criteria for newborn screening. Within that there's always a broad variation of normal and so we can't speak necessarily to what the real cut offs are, what the real curve looks like in terms of the normal population.

We know also that the TREC drops pretty quickly during that first year of life. This graph is taken from a paper actually by Kate Sullivan at [Cha 00:18:46] where they looked at a population of patients with Sjoren's syndrome and the black diamonds are normal controls. You can see that there's a couple of them that were in the first year of life, although it's not clear exactly how old they were whose TREC were very high. This is expressed as the number of TRECs per microgram of DNA rather than per microliter of blood. A little bit hard to make that conversion, but one can see that the next dots are kids who are presumably about five to seven years of age and they are maybe half or so of what that ones are in neonate. You see that they drop off pretty

rapidly in the first few years of life, but when does that drop off occur? Does it occur at two months? At six months? At nine months? That we really don't have good data on. Next page, next slide.

The normal values we really don't have a good idea. There were some... There's a couple small [inaudible 00:19:56] that looked at this for HIV, but again the pat... The studies only have small numbers of patients and they don't do a good job of stratifying them as finely as we would need to see like a baby at birth and then a baby at one month, two month, six months, etc.

To know how quickly this drop off occurs and at what point do we really need to get concern for these older babies or should we even do advocate doing TREC for a baby after say nine months of age. We don't know the answer to that. I don't know... Since Georgia is not a state that keeps its dry blood spots for a length of time, I know there's some states that do. They might have the ability especially if they have those circumstances where a spot is sent to them because of a clerical logistic era of error and they want to do the newborn screening. The TREC comes back abnormal they might actually have the original that they could go back and look at it.

I honestly don't know how often that happens in a given state. It hasn't happened much yet in our state, but we don't know if we should be telling our screening people well don't even bother doing the TREC if they're over say nine months of age. That's an issue that probably we need to think about and need to work on resolving. I think that's... I think that's it. Yeah, okay. That's really the two main issues that I wanted to bring up today. I'll turn it back to Ruthanne to open up for discussion and see if anybody else has any experience with this. Whether they have any ideas about how they handle it.

Ruthanne Salsbury: Great, thank you so much Dr. Kobrynski. We really, very much appreciate your time and for sharing your [inaudible 00:22:01] experiences in research with us. I'd now like to open up all the lines. I'm going to give it a shot with unmuting everyone so that we can just engage in a discussion. If there is too much background noise, we'll just have to do the star seven method. Just give me one second I'm going to unmute the lines. All right and then for those of you that have questions or comments please feel free to share them at this time.

Lisa Kobrynski: I know that someone had a question kind of see about the transfusions. Is there anyone from Tennessee on the line?

Ruthanne Salsbury: Yes. Unfortunately she wasn't able to join the call, but I'm happy to share her question with everyone. Her question is, what is your stance on transfusion? Does this affect TREC results? Are there certain types of transfusions platelets, whole blood, or plasma that require reflection?

Lisa Kobrynski: I mean I can tell you what we... We've talked about this a little bit and my... I've talked to people in California and Wisconsin about this. I can say that in a term baby I think it's very unlikely that a transfusion is going to affect it. Our standard practice in our

state and I think in many states is that all, but very young babies are going to get blood that is depleted of white blood cells, so it's leukodepleted.

Yes you're going to slightly deplete or slightly dilute the white blood cell count, but probably not enough to make your TREC become abnormal. I think the amount of volume to the [inaudible 00:23:49] that you give with plasma or platelets is not going to be enough to dilute your TREC. It's a different story if you do an exchange transfusion. Sometimes you get a baby with hemoglobin [inaudible 00:24:03] that they have to basically exchange the blood volume of the baby. In that situation, which is pretty rare.

Yeah I mean you're... You're usually repleting it with a depleted you know white blood... Packed... Sorry not packed, whole blood that is somewhat depleted of white blood cells. The same thing can be true after they undergo cardiac bypass surgery because they're having a heart repair because the whole blood that they use for the pump is usually depleted of white blood cells. Those are two instances where I would wait, but otherwise no. The question we had with these very, very premature babies that are really getting transfusion awful lot... How much is too much that is going to actually affect your TREC.

Ruthanne Salsbury: Thank you so much. For everyone, these calls are recorded and they're archived on our website. If you know anyone that might be interested in some of the topics that were addressed today please feel free to share the link to this webinar so that we can continue to share this information with everyone in the community.

Does anyone else have any questions or comments? Please feel free to type them in the box on the bottom left or you can just say them over the phone line.

Jennifer Taylor: Hi this is Jennifer Taylor from RTI.

Ruthanne Salsbury: Hi Jenn.

Lisa Kobrynski: Hello.

Jennifer Taylor: Hi, how are you?

Lisa Kobrynski: Good.

Jennifer Taylor: I had a comment. I think Dr. Kobrynski brought up some very good questions and I was just wondering if any other states had any comments. Specifically about the comment about testing older specimens or specimens from older infants and do they see significantly lower values on those.

Ruthanne Salsbury: Please feel free to share your state's experience. I'm happy to read it out loud too if you'd like to type it in the chat box.

Lisa Kobrynski: I know we might have to get Marcy to send out a request to different states for some data because it may be that you know, people may not know off the top of their head how often that happens and that's understandable. We've been paying a lot of attention to it because we're relatively new to starting this in a state that's been doing it for a lot longer may not just have that information off the top of their heads.

Ruthanne Salsbury: Very true, so I am happy to pass that request along to our data team and we will be happy to look into that. I also received a question to remind everyone of the website where you can see which states are currently screening for SCID and that is at www.newsteps.org/scid. There's a map there and there's also a breakdown of each of the states and their current status.

All right, do we have any other questions or comments for Dr. Kobrynski? Dr. Kobrynski did you have any questions that you'd like to pose to the community?

Lisa Kobrynski: No I mean like I said I'm... I mostly just wanted us to maybe start opening up a way of gathering a little bit of information about this stuff because I do think that this is kinda thing collectively can be very useful. Especially to states that are starting screening you know and is relatively new for them.

Ruthanne Salsbury: Definitely, definitely. All right well I am not receiving any additional questions or comments. I am happy to reach out to everyone that's been on this call and just the broader community for the information that you've requested during this call. I think that it'll be really interesting to move forward with some of this information and continue to collect data from states.

If you have any questions following this call please feel free to reach out to me. You can email me at Ruthanne.salsbury@aphl.org. I want to thank everyone for your time. We very much appreciate it. This call will be recorded and available on our website. Our next call will be on Monday August 1st and it will be back at a regular time at three o'clock PM eastern time. As always, please feel free to submit topics for discussion and we welcome presentations from groups and effort, so... Thank you all so much and hope you have a wonderful rest of your day.