BBGuy Essentials 072CE:
Neonatal Platelet Transfusions with Martha Sola-Visner
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Martha: Hi! I am Martha Sola-Visner, and this is the Blood Bank Guy Essentials Podcast.

Joe: Hey everyone, welcome to Blood Bank Guy Essentials, the podcast where we just cover the essentials of Transfusion Medicine. I am your host and my name is Joe Chaffin. Today, we are really fortunate to have a brilliant neonatologist, Dr. Martha Sola-Visner on the podcast to tell us if everything that we've thought about transfusing platelets to babies over the years has been completely wrong!

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So one of the most emotional situations in any hospital is when babies are in trouble. I think we can all agree on that. People tend to do everything that they can to try and keep those babies safe. And I think that's really a good thing. Now, premature babies, especially those with really low birth weights, are definitely at risk for a lot of problems, including the fact that they tend to have really low platelet counts, and they tend to bleed. And sadly, sometimes those bleeds are in the baby's head and things don't tend to go well.

Well, traditionally, when a baby starts to drop their platelet count, neonatologists have just transfused platelets. The platelet count that serves as the threshold for that transfusion decision, though, has really varied over the years and from hospital to hospital and country to country, really. And my guest today, Dr. Martha Sola-Visner from Boston Children's Hospital, has spent much of her career just studying how her fellow neonatologists transfuse babies with thrombocytopenia. And Martha is here to share some surprising information from a study that was published earlier in 2019 by Anna Curley and Simon Stanworth and their group, in the *New England Journal of Medicine*. And that article is really shaking things up in neonatal hematology. And by the way, you can find links to that article, from the *New England Journal*, as well as Martha's editorial that was published in the same issue, on the show page for this episode at BBGuy.org/072.

So my guest, Dr. Martha Sola-Visner is, as I mentioned, a neonatologist. She is an Associate Professor of Pediatrics at Harvard and Boston Children's Hospital, and Director of Newborn Medicine Clinical Research at Boston Children's. She's board

certified in neonatal/perinatal medicine, and she serves as an editorial board member for the journal *JAMA Pediatrics*.

Martha has spent her career focusing on studying neonatal hematologic disorders with an emphasis on platelet disorders, in fact. Her lab has been responsible for breakthrough studies describing differences between adult and neonatal platelet production and lifespan. And her recent work has shed some really new light on the problems that can ensue when adult platelets are transfused to neonates. Martha has authored or co-authored over 70 publications and peer-reviewed journals, and her research has been supported by numerous NIH and NHLBI grants throughout her career.

So, are you ready to challenge some cherished assumptions about platelet transfusions for babies? I hope so, because here's my interview with Dr. Martha Sola-Visner, on neonatal platelet transfusion.

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Joe: Hey, Martha, welcome to the Blood Bank Guy Essentials Podcast.

Martha: Good morning, Joe. How are you? I'm so happy to be here. Thanks for the invitation.

Joe: It's my pleasure. I'm super-excited to get the chance to talk about neonatal platelet transfusion with someone who's passionate and has such a great degree of expertise in this. And Martha, I've already told people all about the wonderful stuff that you've done in your career, and I'm really astounded. You've done such a great amount of not only basic science research, but you've been heavily involved in clinical and translational research on neonatal platelet transfusion. So I have to ask, where did that passion come from? How did that become your "thing," Martha?

Martha: Oh, that's an interesting question. Sometimes the stars and destinies just align correctly. Ever since I went to medical school, which I completed in Ecuador, where I'm from, I loved hematology, and I particularly loved platelets for whatever reason. All my life, I had wanted to be a doctor and do research, but in medical school I really enjoyed hematology. So I took the tests and applied to residency program in pediatrics in the United States, and I entered residency with the intention of following it up with a hematology fellowship. So I came in loving hematology.

Once I actually started doing my residency, I discovered that hematology came with oncology. Emotionally, I could not handle oncology. So I needed an alternative path. And it just so happened that I also discovered that I loved the neonatal intensive care unit, and that I clinically really enjoyed taking care of babies and being in an intensive care unit. And exactly the year that I started my residency, Dr. Robert Christensen, who is the "father of neonatal hematology," had joined University of Florida, as the chairman of Pediatrics, and he was my first attendant in the neonatal ICU. I mean, this really is the ultimate "star aligns."

After working together for a month, he invited me to his laboratory to do research. And that same year, thrombopoietin, which is the main stimulator of platelet production, was discovered for the first time, isolated and cloned. So he asked me to work on platelets because that's the one thing his laboratory wasn't doing. They were doing red cells, they were doing granulocytes, and they were not doing platelets. So it was an unbelievable opportunity for me to do what I love clinically, but yet do research in this one thing that for whatever reason, had always interested me in hematology. And that's how it all started. I've done a lot of different types of research, but it's been very focused on platelets and neonates.

Joe: I want everyone listening to hang with us for just a second because I'm going to ask three questions that Martha has put together that I think are really important for us to think about. And we're going to answer those questions as we go through this talk. First question. They're actually all **true/false questions**. So I'll give you three statements, and you decide in your head whether they're true or false.

The first statement is: **The degree of thrombocytopenia is an accurate predictor of bleeding risk in neonates**. So think in your head, true or false on that one.

Second, **neonates have inadequate primary hemostasis due to their platelet hyporeactivity**. Again, very important. Keep that one in mind.

And number three, **platelet transfusions decrease the risk of bleeding in thrombocytopenic neonates**.

If we get the answers to all those three questions, Martha, I think we're going to be in good shape at the end of this. I'm pretty sure you know those answers, but I won't ask you to give them right now. Okay?

Martha: Sounds great. Sounds good.

Joe: Let us again set the background on this. I think it's very important for my learners and my listeners to this podcast to get a real clear picture of what it is that we're talking about here. In other words, Martha, I would love for you to start by just telling us, how common IS platelet transfusion in newborn babies? Do we have any data on how frequently newborns get transfused? And further, is there any particular risk factors that will lead a patient, a baby, to be more likely to get a platelet transfusion?

Martha: Yes. Neonates are actually one of the most heavily transfused populations in the hospital, both for red cells and for platelets. In terms of platelets in particular, the incidence of thrombocytopenia in neonates who are admitted to the neonatal intensive care unit is about 18 to 35%, depending on the study. So almost a third of babies, of all babies in a neonatal ICU, will develop thrombocytopenia at some point during their stay. And we also know that the smallest and most premature babies are the ones that are most susceptible to developing thrombocytopenia. And they are also the ones that receive the most platelet transfusions. We have some data from University of Iowa that has been shared with us, not

published, showing that in babies who are less than one Kg, approximately 36% will receive a platelet transfusion, and the majority of them will actually receive two to three in the course of their initial hospitalization. And the reason for that is that not only are these babies very susceptible to developing thrombocytopenia, but they also are the patient population that has the highest incidence of bleeding of any other patient population in the hospital. So 25 to 30% of babies who are born with a weight less than 1500 grams will have an intracranial hemorrhage that is most commonly an intraventricular hemorrhage. So about a third of babies will have an intracranial hemorrhage. These hemorrhages is almost always happen in the first week to 10 days of life. And they also happen in babies who do not have thrombocytopenia. So the causality of a relationship between thrombocytopenia and the development of these hemorrhages has been very questionable.

Joe: I see. Just again, for those who don't deal with this on a day-to-day basis, those of us who aren't like you, an neonatologist, when you say that a baby has an "intraventricular hemorrhage," what is the outcome of that? I mean, obviously, it sounds awful, but what are the potential outcomes of a baby having an intraventricular hemorrhage?

Martha: That's an excellent question. It depends on the severity of the hemorrhage. There are different severities. The mildest ones, grades 1 to 2, they may have no significant consequences. But those that are more severe, which we categorize as grade 3 or 4, they definitely have consequences. And they tend to happen in anywhere from 10 to 13, 14%, depending on institutions and statistics of babies. And those are almost always associated with neurodevelopmental consequences that can range from motor deficits like cerebral palsy to developmental delays.

Joe: Okay, well, obviously that sounds terrifying. And I think as a result of that, over the course of my career as a blood bank physician, I have certainly gotten the gestalt from physicians in neonatal ICUs that neonates need higher platelet transfusion thresholds. In other words, the numbers that we talked about for grownups, those are grownup numbers. The babies, we really need to work hard to keep their platelet counts higher. I'm wondering, Martha, has there been any data accumulated about that over the years? I mean, again, that's been my FEELING, but is there evidence? You guys have any evidence that that's how neonatologists feel?

Martha: Yes. So you are absolutely right. The fact that this population is so fragile, and has such a high incidence of thrombocytopenia, and has such a high incidence of bleeding, with such important lifelong consequences, of course, has led neonatologists to try to do anything in our power to prevent these hemorrhages from happening. And it has been widely assumed that babies need, like you said, higher platelet counts in order to prevent bleeding, and therefore they are transfused at high thresholds.

So about 10 years ago when I started becoming interested in platelet transfusion practices (and this was just seeing that we had very little evidence and that people seemed to give platelets a little bit without rhyme or reason in the neonatal ICU),

we decided to actually explore what neonatologists were doing. And we sent a survey to 2700 neonatologists in the United States that were members of the American Academy of Pediatrics, neonatal-perinatal section. We received about 1000 responses, a little over 1000 responses. The way that our survey was, is they sent these neonatologists 15 different clinical scenarios from premature babies, full-term baby, sick, not so sick, young, not so young. And we asked them at what platelet count threshold, below what platelet count, would they transfuse this baby if the baby developed thrombocytopenia in this particular scenario? And we gave them choices that ranged from less than 10,000, so really restricted, to less than 150,000.

The surprising thing to us was that in every single case scenario, every single choice was selected at least once, meaning that the variability was astonishing, from neonatologists allowing babies to get to 10,000 before transfusing, to others transfusing below 150,000 in every case scenario. In most case scenarios, less than 50,000 was the preferred threshold. But if the baby was sick, usually about half of neonatologists would transfuse for less than 75,000 or less than 100,000. So we discovered that, yes, there was a tendency to transfuse at really high levels that I don't think you would use in adults or even in other children, and that there was an extreme degree of variability.

And then we actually translated this same survey and sent it to the directors of NICUs in German-speaking European countries, so Germany, Austria, and Switzerland. We presented them with the same case scenarios. And what we found is that they had significantly more restrictive platelet transfusion practices in almost every scenario. They were transfusing at lower levels. So this suggested to us that North America neonatologists transfused at more liberal thresholds, than European neonatologists.

Joe: I see. That is very interesting the differences and a little frightening with the variability. But Martha, come on now, I mean, those are surveys, right? I mean, people can say anything on a survey. I'm sure you guys had those thoughts that, "Okay. People may SAY that they'll do this, but what actually happens in real life?" Do we have any data to actually look at the behavior either in Europe or in the United States of those neonatologists and see exactly where they ARE actually ordering platelets?

Martha: You're absolutely right. That's exactly what we thought at the time, "These are surveys!" So we went on to get funding to perform a retrospective cohort study where we looked at almost 1200 infants, we call them "very low-birth-weight infants." Those are babies that are born at less than 1500 grams, at the time of birth, and we looked at almost 1200 very low-birth-weight babies in nine NICUs at four different academic institutions over the years 2006 to 2007. So around the same time that we performed the survey. But I want to highlight that this was a little over 10 years ago. So what I'm going to talk about are the practices then, but it's an accurate reflection of what U.S. Neonatologists have been doing.

We looked at almost 1200 platelet transfusions. And what we found was that 28% of those transfusions, those almost 1200, were given in the first seven days of life. Showing that there is a lot of fear in that time when the risk of intraventricular hemorrhage is also highest. And 83% of those transfusions were given for a platelet count greater than 50,000. Anywhere from less than 150,000 to 50 to 60. So it is true, 83% of all transfusions were actually given for platelet counts greater than 50,000. And we then looked at the other 72% of transfusions that were about 856 total transfusions that were given to infants that were older than seven days. And 51% of those were still given for platelet counts greater than 50,000.

So what we learned was that the survey probably reflected the truth in terms of selecting high platelet transfusion thresholds for this very high-risk population of infants. And we also looked at a similar study that was a prospective, observational study of thrombocytopenic babies that was published by Simon Stanworth in *Pediatrics* in 2009 [NOTE: Link at BBGuy.org/072], looking at the same question in the United Kingdom, and although I don't have the statistics, it is probably less than six or 7% of babies who are transfused for platelet counts grade than 50,000. So a much smaller percentage.

Putting this data together, I think it is true that there is a tendency to use more liberal platelet transfusion thresholds in neonates in the United States compared to European neonatal ICUs.

Joe: Well that seems clear certainly from that data. There is one thing that just occurred to me, Martha, as you're talking about that. It's interesting to me... And this is just an aside, I hope you'll you don't mind me taking a little side trip for just a moment. But in your study that you mentioned from 2016... And by the way, everyone, I should have said this at the beginning. The studies that Martha and I are discussing will be linked on the show page at the Blood Bank Guy website at BBGuy.org/072. So you can find the references to these articles that Martha and I are talking about. But anyway, back to this study in *JAMA Pediatrics* 2016, you had mentioned the main points that in the babies less than seven days of life that 83% of those transfusions were for babies with platelet counts over 50,000.

But it's interesting to me how many MORE platelet transfusions occurred in the babies who were older than seven days of life. 72% of the transfusions in your study happen for babies older than seven days. Only 28% less than seven days. That is curious to me. I mean is that... You know more about the dynamics of neonatal platelet production than I do by a million miles. Is that a typical thing? Do we see more of a drop later on for these babies?

Martha: That's an excellent question. The reason this happens is because babies that are born so prematurely stay in the NICU for months. I mean, the length of the NICU stay is usually anywhere from two to four months. So until they are about full term. So baby who's born at 24 weeks and will stay with us until he is 40 weeks, then that's going to be a four-month stay just until they're ready to go home. During that time, there are many reasons for thrombocytopenia. There are many different diseases that can cause thrombocytopenia. Some of them present in the first week

of life, but many of them, especially sepsis, and then a devastating disease called "necrotizing enterocolitis" present after seven days of life. That's late-onset thrombocytopenia.

So, of course, there is a very high risk of having one of those after one week since you have an additional 14, 15 weeks of hospitalization. I think what this reflects is simply the distribution of the onset of thrombocytopenia, and some of them being early on, and some of them being late on. And giving the length of these periods, obviously, there is a higher likelihood of having thrombocytopenia later on.

Joe: That makes total sense. That probably should have been obvious to me, but I missed that. Okay, I totally understand. So let's move on Martha. And let's ask what I think is a really really, really important question. We've talked about how platelets are transfused and the thresholds, whether formal thresholds or informal thresholds in neonatologist minds, that are being used in the real world, or at least were a few years ago. So I think it's a very important question to ask and to see if there's any data on figuring out is there any correlation between the platelet count and the risk of bleeding? Is there any work that's been done on that?

Martha: Yes. The answer is actually fascinating. Because when we looked at the risk of IVH, the development... And again IVH stands for an intraventricular hemorrhage. When we looked at the correlation between IVH and thrombocytopenia, defined as a platelet count less than 150,000, we found that those babies who were in the first week of life only had a platelet count less than 150,000 had a risk of IVH that was 2.3 higher compared to the babies who never had a platelet count less than 150,000. So there IS an association between having a platelet count less than 150,000 and having a higher risk, almost a little over a two fold risk, of developing IVH.

However, when we then looked at the babies who had platelet counts less than 150,000 and looked and asked the question of whether the risk of IVH increased with worsening severity of thrombocytopenia. In other words, having a lower platelet count increases your risk more than if the platelet count is 140,000. The answer was no. Within that group, there was no correlation between how severe the thrombocytopenia was and the actual risk. The risk was the same, independent of the actual platelet count.

This was a retrospective study. But Simon Stanworth, again, in his article in *Pediatrics* in 2009, it's a wonderful article that I would recommend, performed a prospective observational study where they had 169 babies with platelet counts less than 60,000. And he did the same thing, correlated the lowest platelet count before bleeding, or the lowest platelet count ever with the degree of bleeding classified as non, minor, or major. And he found the same thing, that there was no correlation really between having more severe thrombocytopenia and having more major bleeding.

And the same thing has been seen in pediatric and actually adult patients, where there is no good relationship between the morning platelet count in patients who

are undergoing chemotherapy or bone marrow suppression therapy and have thrombocytopenia as a result of this myelosuppression and the risk of bleeding. You know, it does go up when the platelet count is less than 100,000. But then it's about the same until the platelet count is less than 10,000, where it really, really increases. So, this seems to not be unique to neonates.

Joe: That's really interesting. And that study that you're mentioning, I believe, is in a sub-analysis of the famous "PLADO" study that Cassandra Josephson led in 2012. Is that the article you're referring to Martha?

Martha: That's exactly the article I'm referring to. Correct. Thank you.

Joe: Oh, that's a spectacular article. And it solidified the fact that I'm a huge fan of Cassandra. But nonetheless, that goes without saying, but...

Martha: I am too.

Joe: We will make sure that that article, by the way, everyone, is linked on the show page again at BBGuy.org/072.

So we're sitting here with a conclusion that, well, thrombocytopenia is associated with bleeding (and please correct me if I'm not stating this correctly), that thrombocytopenia is associated with bleeding and is of course associated with intraventricular hemorrhage in babies. But you can't really tell risk for the baby based on how thrombocytopenic they are. Is that an accurate way to put it?

Martha: That is correct.

Joe: I think that is surprising. And that is surprising to most people who would hear that that haven't looked specifically at those articles. What kind of either research questions or just general questions does that bring up in your head?

Martha: The natural conclusion is, well, if we maintain the platelet count above 150,000, and don't allow a baby to become thrombocytopenic, can we then decrease the risk of bleeding? In other words, do platelet transfusions in these babies in the first week of life, decrease the incidence or the severity of IVH? And this question actually was addressed in a study that was published in 1993. This study in 1993 by Maureen Andrew, was a landmark study and up until very recently, the only randomized control trial of platelet transfusion thresholds in premature babies. And what Maureen Andrew and her group did is they asked exactly that question, can we just keep the platelet count normal and prevent IVH? So they randomized 152 very low-birth-weight babies in the first week of life, they were all less than seven days, and randomized them to receive platelet transfusions for any platelet count less than 150,000 or allowing them to drop to less than 50,000 unless there was clinical bleeding.

What they found at the end of seven days, is that there was no difference in the frequency or in the severity of intraventricular hemorrhage. So the group that was aggressively transfused had an incidence of 28% of IVH, and the control group

that was allowed to drop to 50,000 was 26%. This study answered that initial question and show that transfusing very low-birth-weight babies with platelet counts that are greater than 150,000 in the first week of life does not reduce the incidence or the severity of IVH.

Joe: So as you mentioned, that was the only study of its kind that we had until recently until the study that I mentioned at the beginning. And we're going to get to that study in just a few minutes, the Dr. Curley study. But before we get there, I think there's one more question that we have to ask. Perhaps there's data on this, I'm guessing that there might be. I think it's a super important question. You've talked about whether or not platelet transfusions help with decreasing intraventricular hemorrhage. Do we know anything about whether those platelet transfusions actually help to save babies lives? Does it decrease neonatal morbidity and mortality?

Martha: Again, an excellent question. Up until the study by Dr. Curley, very recently, a number of retrospective studies tried to address this question, are platelets helping? And we need to understand that thrombocytopenic babies are sick babies. So it's very hard to disentangle the confounding effects of severity of illness in any retrospective study. Nevertheless, our group looked at this question, and we found in a couple of studies in different populations in the US... One study was in the US and other one was in Mexico, that the neonates who receive platelet transfusions had a relative risk of death that was 10 times higher than that of non-transfused neonates. So linking transfusions to mortality. But again, it's hard to say whether there's a severity of illness component.

Dr. Christensen, again, my mentor in Utah, he performed a study of 1600 thrombocytopenic neonates. And again, found that the number of platelet transfusions predicted the mortality rate, and he performed a statistical sensitivity analysis to try to sort out the effect of severity of illness. This analysis suggested that some of the mortality was actually due to the harmful effects of platelet transfusions themselves.

There have been another couple of studies looking at necrotizing enterocolitis, a devastating disease in neonates that is associated with severe thrombocytopenia. One was by Dr. Kenton et al., in 2005 that showed an association between more platelet transfusions and more development of short bowel or cholestasis which are complications. And there was a study published by again, our group, in combination with Cassandra and with Ravi Patel at Emory. This was a secondary retrospective analysis of a multicenter prospective cohort study of very low-birth-weight babies at the three centers in Atlanta. We had 44 infants who developed necrotizing enterocolitis, and we found that the platelet transfusion rate was 35 per 100 infant days among infants who died, compared to 4.9 per 100 infants days among the survivors. So huge difference with those that died receiving more platelet transfusions.

And in this study trying to address the issue of severity of illness, we adjusted for birth weight and illness severity at the onset of NEC and found that the platelet

transfusion rates after adjustment were still three times higher in those babies who died versus in the babies who did not die. And even though the adjusted risk ratio did not reach statistical significance, it was three times higher in those babies who went on to die than in those babies who didn't die. We think this cohort was very small, but there was a very strong suggestion that babies who receive more platelet transfusions had a higher risk of death than those who did not, even after adjusting for birth weight and illness severity.

Joe: Well, those are scary numbers. And as you said, that it does raise a lot of questions and it kind of leaves us with a few things tha,t granted, these are retrospective studies, as you mentioned, and that challenges being retrospective. But I think that brings us to a little bit where we started, as I mentioned, the article that was published in the *New England Journal of Medicine* in January of 2019, Dr. Curley, and I would be remiss if I didn't mention Dr. Stanworth also on that, titled "Randomized Trial of Platelet Transfusion Thresholds in Neonates." As I mentioned, Martha, you did a wonderful editorial commentary on this study, and I would love it if you would just take us through generally how they set it up, generally what they were looking for, and what they found.

Martha: Yes, sure. So this was a landmark, long-awaited study, published by Dr. Curley and Stanworth as co-first authors, equal contributors. This was a randomized control trial that enrolled neonates with a gestational age less than 34 weeks. So they were all premature, who had a platelet count less than 50,000, and were randomized to receive platelet transfusions for any platelet count less than 50,000 or only if the platelet count dropped below 25,000. So 50,000 versus 25,000 as the liberal and restrictive thresholds.

The primary outcome was death or major bleeding within 28 days following randomization. And the bleeding was quantified using a standardized quantitative tool that was specifically developed for neonates and that had been previously validated and publish for neonates.

So the population that was involved in the study was very representative of your typical NICUs. These were small babies that were born at 26 weeks of gestation, because those are babies that most frequently developed thrombocytopenia. They had a median birth weight of about 740 grams at birth, very small, less than 1000 grams. And the median postnatal age at randomization was seven days. And I will also point out that approximately 60% of babies in the study, were receiving antibiotics for sepsis. So indicating the importance of sepsis. The almost surprising finding was that death or major bleeding until day 28 post-randomization, the primary outcome, showed that the incidence of bleeding or death was actually statistically significantly HIGHER in the high threshold group in those babies were transfused at less than 50,000 compared to the lower threshold group. So again, those babies who were transfused at higher platelet counts at 50,000 had a higher incidence of bleeding or death by 28 days after randomization, compared to those who were allowed to drop their platelet count less than 25,000.

So, in general, this "PlaNeT-2 study" that is the highest level of evidence that we have so far showed higher bleeding and mortality among preterm neonates randomized to liberal platelet transfusions. And I'm going to say that while this results seemed surprising, they were actually consistent with all the body of evidence and the prior studies that I have shown you and I've discussed on the show, that showed that there is really no good correlation between platelet counts and bleeding, that have shown that there was no evidence and at least retrospectively, and in the one single prospective study we had, of platelet transfusions in reducing bleeding. And that had also suggested this nagging association between the number of platelet transfusions and higher mortality and morbidity in premature babies.

So even though they seem surprising to the field, they were actually consistent with this growing body of evidence that had been accumulating in the last decade.

Joe: Martha, thanks for summarizing PlaNeT-2. As you said, I think that kind of confirms a lot of the stuff that we were concerned about and we were thinking. I do have to ask, though, in randomized trials like this, where you have so-called "liberal" and "restrictive" strategies, I often wonder if there is data about how well people followed those guidelines. In other words, were there places where they didn't transfuse where they should have or shouldn't have transfused and did, things like that? And further, is there anything else in this study that just kind of makes you raise your eyebrows and go, "Hmm, that's interesting."

Martha: Yeah. In general, they had pretty good adherence to the thresholds. It was...I don't have the data in front of me, and I don't know the numbers of heart, but I'm sure this study will be linked to your podcast or I hope so, but they had in general good adherence. I think one of the problems that raised concerns among neonatologists, however, this just comes to issues that sometimes happen in randomized control trials, is that 39% of the patients enrolled in the study had received one or more platelet transfusions before the randomization. So again, that's a high percentage of babies, who for reasons that were not specified in the paper, had received at least one platelet transfusion prior to entering randomization. So this raises a little bit of the concern whether these babies... At what platelet counts were these babies transfused? Why were they transfused before randomization? Was this during the very high-risk period on the first seven days of life, or was this when the babies were sicker? We hope that some follow up analysis will provide some of these answers so that were not provided in the PlaNeT-2 study.

Joe: I was curious about this when I saw what they had said about "major bleeding" what they meant by that. And again, please correct me if I'm wrong, but I think what I saw in the article was that major bleeding included not just intracranial hemorrhage, but really significant bleeding of any kind. Not just in the brain in other words. Is that correct, Martha?

Martha: That is absolutely correct. And it's actually very important to point out. This study was not restricted to intraventricular hemorrhage, and it was not restricted to the

first week of life. It actually looked at the entire hospitalization. And it had multiple different types of bleeding that could represent major bleeding, if they required, for example, changes in the ventilator if there was a pulmonary hemorrhage, or if they required red cell transfusions or fluid boluses by causing hemodynamic instability, so there were multiple types of bleeding that were assessed. But they were very clearly defined because they used a quantitative and previously validated neonatal bleeding assessment tool to quantify bleeding every single day for these 28 days after randomization.

Joe: Perfect. Okay. Thank you for clearing those two things up. So Martha, this is the part where the basic scientist in you has to be... your heart has to be swelling, because I think it's really important. I mean, we have this data, we have this randomized trial that's showing something that, as you said, is consistent with the prior studies and consistent with some of the hypotheses that you would have come up with from your basic science work. But I would love for you to share a little bit from not just the work you've done in your lab, but in anyone's lab. Why do you think this could be? First, I think the place to start, what do we know about how well platelets work in babies?

Martha: Ah, another excellent and fascinating question, Joe. So yes, the question we're left with now is, "Why? What are platelet transfusions doing that is increasing the mortality?" You're right. The place to start is, what do we know about baby platelets? Interestingly, and this may be has contributed to the belief that babies need to be transfused at higher platelet counts, multiple studies over the last two decades have shown that platelets even in full-term babies, but also in preterm babies even more so, are hyporeactive to nearly all agonists, at least in cord blood, and certainly for the first 10 days of life. So studies using platelet activation, whole blood platelet aggregometry, have shown that if you compare neonatal and adult platelets stimulated with multiple agonists, thrombin, TRAP, ADP, epinephrine, collagen, they respond much less, they activate much less than adult platelets.

Surprisingly, however, Maureen Andrew, already in 1989 showed that bleeding times are shorter, SHORTER in healthy full-term babies than in healthy adults. So, of course, we know that bleeding times are subjective, they depend on the skin thickness and they're operator dependent. So there was a little doubt methodologically cast on those results. However, at least four subsequent studies that have used an in-vitro measure of primary hemostasis that is supposed to be equivalent to the bleeding time, the platelet function analyzer closure time, which is non-operator dependent, is done in a machine and measures the time that it takes flowing blood to occlude small aperture when stimulated with collagen and epinephrine, or stimulated with collagen and ADP.

So those studies, all of them show that neonatal cord blood had SHORTER closure times compared to normal, healthy adult blood, indicating that neonatal blood at birth actually has more robust primary hemostasis than normal, healthy adult blood. So how is it possible that you have global platelet dysfunction, yet you have more robust primary hemostasis in babies compared to adults?

The answer to this paradox is that the neonatal platelet hypoactivity is not a deficiency, is not a defect of babies, but it's actually part of a well-balanced, unique neonatal primary hemostatic system that is very different from that of adults, with a very different balance, in which the hyporeactive platelets are completely counterbalanced by factors that actually increase and stimulate clotting, including the high hematocrit that babies have at birth with very high MCV. And the fact that they have high von Willebrand Factor levels and a predominance of ultra-large von Willebrand Factor polymers (resembling what you see in TTP plasma). All these factors completely balance the hyporeactive platelets, and that result is actually a little bit of shorter bleeding times and shorter closure times. It's not just adequate, but actually a little robust primary hemostasis in neonates.

Joe: That's fascinating. So despite the fact that the platelets (I'm just summarizing, because it's very cool information), despite the fact that if you look at the platelets themselves, they don't work as well as adult platelets, everything else balances that out to make you as a baby actually have a more functional coagulation system. That's somewhat surprising to me, Martha, but that's awesome.

Martha: It's absolutely correct. Babies have a high incidence of bleeding, but they also have the highest incidence of thrombosis within the pediatric population.

Joe: That actually brings up another question and you raised this in your editorial, I guess this kind of goes to the heart of the matter: If you have a baby who has a balanced coagulation system, despite the fact that they have hyporeactive platelets, and you toss in "grown-up" platelets on top of that, that seems like that could be a problem. Is that part of your hypothesis as to why this could be an issue?

Martha: Yes, we wondered the same thing and performed a study that was published in the *Journal of Thrombosis and Hemostasis* in 2011, to try to answer this in-vitro. And what we did is we took normal cord blood, full-term cord blood, normal adult peripheral blood, and then we actually used the buffy coat modified method in the laboratory with multiple different spins and separations to generate thrombocytopenic blood, and we dropped the platelet count in-vitro to 50,000. And then we generated miniaturized platelet suspensions, both derived from cord blood and peripheral blood.

And then we performed autologous and allogenic developmentally mismatched, you could call that "miniaturized" in-vitro platelet transfusions. So we mixed adult platelets with cord blood and neonatal platelets with adult blood. Then we checked whether the platelets placed in a different environment still retained their activation, their properties from the original blood. We used whole blood aggregometry to do that. And indeed, the neonatal platelets were still HYPOreactive when they went in the adult blood, and the adult platelets were still HYPERreactive, comparatively speaking, when they were placed in the cord blood. So showing that these are cell intrinsic properties, there is not an inhibitor in neonatal blood.

But then the most important thing is we looked at primary hemostasis after doing these in-vitro transfusions. What we found was what we had hypothesized: Using the PFA-100, an in-vitro measure of primary hemostasis, we found that the "CT-EPI closure time," so a measure of primary hemostasis in response to collagen and epinephrine, in this test the cord blood that had been transfused with adult platelets, mimicking what happens in-vivo, had the shortest closure times. So the most robust primary hemostasis of all samples, and the adult peripheral blood that had been transfused with a neonatal platelet had the longest ones. So this suggested to us that, yes, the addition of adult platelets into neonatal blood could actually result in a little bit of a prothrombotic phenotype, because of this developmental mismatch. And it is possible that the addition of adult platelets in-vivo also leads to this prothrombotic phenotype and contributes to microthrombosis, that might be one of the mechanisms explaining the findings.

Joe: So, Martha, there's a whole lot more that you as a basic scientist could tell us about hypotheses about other impacts that adult platelets could have. I wonder in the interest of time, if you would just tell us a little bit about what we know and what we think and what we might hypothesize about the inflammatory effect of adult platelets on these neonatal environments.

Martha: There is actually not much data. This is all hypothesis at this point. All we know from the last decade is that platelets are not just hemostatic cells. We know that platelets play a major role in immunity as regulators of immunity and as central mediators of inflammation. And we have basic science data that would lead you to hypothesize that adult platelets have a higher proinflammatory potential compared to neonatal platelets.

I told you earlier that in the study by Dr. Curley, 60% of the babies were being treated for sepsis. So the question we haven't answered that is a valid hypothesis is what happens if you have a septic environment, and now you add platelets that are, and we don't know that, that are potentially more pro-inflammatory? Adult platelets that might be more pro-inflammatory? Are we actually exacerbating the inflammatory response, and could this be a mechanism in neonates? And we don't have the answer to this question. This is a hypothesis. But I think, another potential path that mediates the clinical findings.

Joe: Martha, this has been really enlightening, and I know that people are going to learn a lot from this. But I want to circle back around to the three true/false statements that we threw out at the beginning and give you a chance to give the answers to those. I think they're probably going to be pretty obvious based on the things that you've said during this interview, but let's talk first about question number one, or statement number one. True or false, the degree of thrombocytopenia is an accurate predictor of bleeding risk in neonates.

Martha: I think there is now abundant evidence showing that that is false. That the degree of thrombocytopenia does not predict bleeding risk in neonates. We need better tests, we need better means.

Joe: Okay, how about number two? True or false, neonates have inadequate primary hemostasis due to their platelet hyporeactivity.

Martha: I hope I have convinced your audience that this is also false. Yes, they do have platelet hyporeactivity, but they do NOT have inadequate primary hemostasis. At least not healthy neonates.

Joe: And the very last one, true or false, platelet transfusions decrease the risk of bleeding in thrombocytopenic neonates.

Martha: Well, I think we are pretty certain now after the publication of PlaNet-2 trial, that if you have platelet counts greater than 25,000, the answer is no, they do not decrease. We don't know how low we can go before we reach a threshold where they might, but we do know that above 25,000 they don't.

Joe: I have a feeling that it's certainly possible that some neonatologists in training might listen to this podcast as well, because of the topic, of course, and because of who you are. I guess my question is this, if platelet transfusions don't really help to decrease the bleeding risk in those thrombocytopenic neonates, as you mentioned over 25,000, then what does? I mean, what options do your fellow neonatologists have in these babies?

Martha: Oh, that is a great question! We don't know if FFP transfusions do, even though there's some studies that haven't shown an effect. And fortunately, IVH is multifactorial, and I think hemostasis probably plays relatively small role compared to the hemodynamic factors, inflammation, fragility of the germinal matrix which is the site where bleeding usually starts in the premature babies and a number of in-vivo factors beyond hemostasis. So I think hemostasis in general might be just one of the multiple factors that contribute to bleeding, and therefore we might have limited power to modify that through our transfusion interventions. This is a fertile ground for study. We don't have a good answer for that.

Joe: Martha, it has been such an honor to have you with me. I am overjoyed at being able to talk to you today. Thank you so much for doing it. Is there anything that you'd like to leave us with before we go?

Martha: Yes, I think the only thing I wanted to say is that, clinically speaking, I think that we should consider 25,000 the threshold proven certainly in babies outside of the risk period. Clinically speaking, and this is something that I'm saying mostly for neonatologists, most neonatologists still have a little bit of concern in moving completely to 25,000 in the first seven days of life. And this is mostly because of the high percentage of babies that received platelet transfusions in PlaNeT-2 to prior to randomization, and the lack of clarity as to whether this happened in this high-risk period. And so I think we're awaiting more information in that period. But I think they are working on another manuscript, looking at types of bleeding, timing of bleeding and so forth.

Joe: Martha, again, thank you so much for being here. It has been such a pleasure and such an honor. Thank you very much.

Martha: Thank you so much, Joe. I appreciate it. And thank you for the invitation. It's been a joy.

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Joe: My thanks again to Martha Sola-Visner for joining me today. I really enjoyed speaking to Martha, and I hope you challenged some of those dearly-held assumptions! Now, as Martha said, it's probably not yet time to make wholesale massive changes in our practice, but this information should really make every blood banker and every neonatologist THINK about their next platelet transfusion for a premature baby.

My thanks also go to my assistant editors, Dr. Daniela Hermelin and my wonderful daughter, Samantha Chaffin. Any mistakes you see are my fault and not theirs!

So, remember, if you are a physician or laboratorian, you can go to www.wileyhealthlearning.com/transfusionnews and get your hour of totally free continuing education credit from listening to this podcast. My thanks for that as always to Transfusion News, to Bio-Rad, who brings you Transfusion News, and to Wiley Health Learning.

Again, the show page for this episode is at BBGuy.org/072. You can find links to the articles that Martha and I discussed in this episode there, as well as more of Martha's previous work; especially really interesting work on how "adult" platelets might impact babies.

I also appreciate all of you that have gone to Apple Podcasts and given this podcast a rating and review. Apple uses those ratings to recommend the podcast to others, so if you haven't rated, reviewed, or subscribed, I would really appreciate it if you have time to do that!

The next episode, coming two weeks from now, is a really interesting interview with David Oh and Mike Goodman from Cincinnati about the steps they took to introduce cold-stored, group O, low-titer whole blood for trauma transfusion at the University of Cincinnati. It's really practical and really fun, so I'm very excited for you to hear it.

But until we meet again then, my friends, I hope that you smile, and have fun, and above all, never, EVER stop learning! Thank you so much for listening. We'll catch you next time on the Blood Bank Guy Essentials Podcast.