



**BBGuy Essentials 070CE:
Preventing Alloantibodies in Sickle Cell Disease with Stella Chou
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Stella: Hi! I'm Dr. Stella Chou and this is the Blood Bank Guy Essentials Podcast.

Joe: Hi everyone, and welcome to Blood Bank Guy Essentials, the podcast designed to help YOU learn the essentials of Transfusion Medicine. My name is Joe Chaffin, and I am, as always, your host. This episode is one I've really looked forward to for quite a while! I'm getting the opportunity to speak to international sickle cell transfusion expert, Dr. Stella Chou, about her surprising findings about antibody formation in sickle cell disease

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So, at least three different times on this podcast, people have mentioned Dr. Stella Chou's 2013 article in "Blood" (which I will [link on the show page](#), by the way) on how, even when we sickle cell patients the best way that we can (by the way, that's usually matching for the Rh C and E antigens, as well as K in the Kell system, and preferably, with blood from African-American donors), despite all that, many patients with sickle cell disease STILL make antibodies! And that, by itself, made me very interested in talking to Stella. But, when she and her esteemed group (which includes "friend of the podcast" Dr. Connie Westhoff!) put out a followup in late 2018 that started to explain the "why" behind those findings, I have to admit, I was hooked. So, Stella is here to enlighten us on where we've been, where we are, and where we're going with matching donors to patients with sickle cell disease in such a way as to prevent those antibodies from being formed in the first place.

So, let me tell you a little bit about Stella before I bring her on: Dr. Stella Chou is an Associate Professor of Pediatrics at the Perelman School of Medicine at the University of Pennsylvania. She practices Pediatric Hematology and Transfusion Medicine at The Children's Hospital of Philadelphia with a particular interest in patients with sickle cell disease (of course). Her work has demonstrated that inheritance of variant blood group antigens in patients with sickle cell disease contributes to their high rate of red cell antibody formation, and that's what we are going to discuss today. She also does extensive research, including heavy-duty work with induced pluripotent stem cells (iPSCs) and primary human cells in an effort to try and identify new approaches to minimize alloimmunization, reduce complications, and improve therapy.

I can't wait for you to hear Stella talk today about preventing antibodies in sickle cell disease. Without any further waiting, here's my interview with Dr. Stella Chou:

Joe: Hey Stella, welcome to the Blood Bank Guy Essentials Podcast!

Stella: Hi! Happy to be here.

Joe: It's so great to have you. You know, I was mentioning to you before we started that I have heard your name so many times over the last three years I've been doing this podcast, in association with some landmark work that you've done in sickle cell disease. So it's just such an honor to actually *talk* to the Great Stella Chou! How cool is that? Right? Come on.

Stella: That is really, really nice. But I think what we're trying to do here is just help our patients and make people aware of what we have to think about when we transfuse our patients with sickle cell disease.

Joe: Absolutely. And I know that you are someone who has not only done a lot of great research in terms of sickle cell disease, but you're actually...in your actual practice, do you actually see "real live patients" with sickle cell?

Stella: I do! I'm a pediatric hematologist, so I see patients in the hematology clinic, so that we see them just for their preventative care or if they have had a recent complication and admitted to the hospital. And then I also see patients in the apheresis unit where, at Children's Hospital of Philadelphia, we have a pretty large chronically transfused patient population who gets their red cell transfusions by erythrocytapheresis. So I see the patients there as well.

Joe: So you have expertise on both sides of this, and that's really something that I think is unique for you. That brings a really great perspective.

So Stella, I wonder if we could just start off just with the very bare basics? I have done a podcast a couple of years ago on really bare bones essentials of transfusion in sickle cell disease. I want to cover a little bit of that same ground, but your research has led you to some really cool places that I want to make sure we get to as well. But for those that are just kind of getting started in blood bank world, those who maybe don't have a ton of expertise, I wonder if you could just start with a really brief discussion on what sickle cell disease is and kind of what are the ways that people with sickle cell disease interact with both blood banks and hematologists, people like you?

Stella: Well, so patients with sickle cell disease have a chronic anemia and some of the complications that occur with these patients is because their red cells basically have an ability to cause micro-occlusions in the capillaries of different tissue beds, like our lungs, the CNS system, their liver, kidneys. So it's really a multi-organ disease. And mostly what we see patients chronically transfused for is to prevent stroke. That's probably the number one indication. But we also have patients who

oftentimes get chronically transfused because they've had splenic sequestration that's life threatening. So they drop their hemoglobin acutely from like say a baseline of 7-8 [NOTE: g/dL] down to 3 or 4 and have to get transfused and treated. Sometimes for patients who have multiple episodes of "acute chest syndrome," where they're having the vaso-occlusion in their lung and it causes hypoxia and anemia, and for those patients who end up being treated in the intensive care unit or require multiple emissions with transfusion, we will recommend that they go on a course of chronic transfusion.

And I think it's important to know that this is a really exciting time for sickle cell disease. You're probably aware that gene therapy is something many, many labs and actually pharmaceutical companies are working on. And not only that, but right now we treat so many patients with hydroxyurea, which increases your hemoglobin F and does help to prevent some of the complications of sickle cell disease, so that others are working on new ways to induce the fetal hemoglobin in these patients. But I think that always we will still see patients who require transfusion, either acutely or they have other reasons why they can't be candidates for other types of therapies, and will still need to be chronically transfused.

Joe: Gotcha. So could you talk just a little bit about the indications for acute transfusion or simple transfusion vs. the type of exchange transfusion or transfusion via erythrocytapheresis that you were mentioning?

Stella: Sure. So most times when we do simple transfusion, it's either because they have some kind of anemia that brings them to the hospital...So the common things that we see are anemia due to **Parvovirus infection**, which can occur slowly and sometimes they don't even notice until they get to a pretty low hemoglobin of multiple g/dL lower than their baseline. And what that does is because the Parvovirus causes sort of a delay in their bone marrow just making new red blood cells, we will have to transfuse them to get them "over that hump," per se. Other times are when patients come with the **acute chest syndrome**, which is like a pneumonia. But in patients with sickle cell disease, that would be called "acute chest syndrome," because it could just be from vaso-occlusion. But oftentimes it's triggered by some kind of infection, whether bacterial or viral. **Splenic sequestration**, which we typically see in children who are younger than the age of five. And occasionally we'll see other causes of severe anemia typically from infection or something else that instigates hemolysis.

I would say for chronic transfusion it's usually **patients who are picked up by early transcranial doppler [TCD] screening**. So we typically see patients who are preschool age, oftentimes, who will get picked up with an abnormal TCD where basically it shows that the velocity of their blood flow in their major vessels of the brain is too "tumultuous," basically. So it gives you a high velocity and that is an indicator that this patient is at **risk of stroke**. That's probably the most common indication.

The other common indications are, as I mentioned, patients who have had complications that are very recurrent and can be life-threatening, like acute chest syndrome and splenic sequestration. Occasionally patients are chronically transfused for very chronic pain, but that's not what we consider a true indication, but that will be something some providers will suggest.

And then in terms of erythrocytapheresis, generally this is the way that we can transfuse patients so that they don't iron overload. So patients can also get simple transfusion where basically you come in and you get an IV and you're just given red cells. Typically for children we do 10 to 15 cc/Kg, and we try to not have the hemoglobin above 10 g/dL for patients with sickle cell disease because we worry a little bit about hyperviscosity. And for adults, usually, it's in the range of one to two units. Again, not causing their hemoglobin to go above 10 g/dL. For patients who don't want to "chelate" with their simple transfusions or there are other reasons why they're not amenable to chelation, we will do erythrocytapheresis because basically we're removing red cells and then providing donor-derived red cells. And the iron balance can be either equivalent or a little bit above or below.

Joe: So just to make sure my audience understands what you're talking about there when you say "are not amenable to chelation," could you expand on that just a little bit?

Stella: So we do chelation for patients who are chronically transfused because if you are simply receiving blood and not removing any blood, we know that **for every mL of packed red cells we're basically loading you with one mg of iron**. So in order to not have iron overload in patients with sickle cell disease or other chronically transfused disorders, we use iron chelation. And basically iron chelation is a way where the medication basically draws the iron out and you excrete it through your normal processes. But typically the body usually just recycles iron in order to make the new red blood cells.

I think that adherence to chelation certainly has improved because of these different formulations of iron chelation, but it still is hard for some patients and some families to always remember to take it and always come in for all the screening labs and all the other screening tests in addition to just their routine follow up.

Joe: So Stella, what you're describing in terms of the transfusion needs of patients with sickle cell, it sounds pretty dramatic. I mean it sounds like these patients get transfused fairly substantially over their lifetimes. Is there anything, and I've covered this a little bit briefly in another podcast, but what are the things that can happen as a result of transfusion that we worry about?

Stella: So mostly the main two things we worry about chronic transfusion is **iron overload** and what that can do to affect your liver and your heart function, most importantly, but can also affect other organs and glands. And then we worry about **alloimmunization**, which is obviously the focus of my work. And this is problematic, because for patients with sickle cell disease, once they become

alloimmunized, it's often found that patients become further alloimmunized. And for some patients, it becomes very difficult to find compatible blood to transfuse them, or it takes longer, and the evaluation in the blood bank takes longer, then we have to request special units from your blood supplier. And so, in an acute setting, if they were to just present to the emergency room, say with very severe anemia that could be life-threatening, we are put in a position where we don't have blood, and significant morbidity and mortality still occurs throughout developed countries where we simply just can't find the right blood for them quick enough.

Joe: Those situations are obviously things that we want to keep from happening. So how have we done that over the years, Stella? What have been kind of the traditional strategies maybe that that blood banks and hematologists have used to try and reduce that alloimmunization?

Stella: So I think judicious use of transfusion is really important. So really, we should think hard when we transfuse a patient with sickle cell disease, whether they need it or whether it's making *you* feel better to just get their hemoglobin up. And then the primary thing that has occurred is to provide "limited extended matching" for patients with sickle cell disease. So whereas you and I would likely only get matched for ABO and RhD, for patients with sickle cell disease starting from approximately the mid-90's, we've matched them for two Rh antigens, C ["Big C"] and E ["Big E"]. And some institutions actually specifically match for c ["little c"], e ["little e"], so all Rh antigens, and for Kell, so K ["Big K"], because those have been historically the ones where they're mismatched the most and are immunogenic, too. And this has significantly reduced the rates of alloimmunization.

So one thing that I think is really important to think about (and is sort of my soapbox) is that oftentimes people will quote what the rate of alloimmunization is and give a percentage. And that's actually very different from place to place. So for instance, at a place that just looks at alloimmunization across say one or two years, they might have a prevalence of alloimmunization that's a snapshot in time of say, 10%. But if you are, for instance, an institution like ours, where we've followed patients for some decades because we have a few patients in our apheresis service who are adults and have transitioned to adult care, but their providers can't provide them with erythrocytapheresis, so we continue to provide that service for them. So, if you look at a very long period of time, our prevalence of alloimmunization is going to be much higher. It'll, say, be 50% for our chronically transfused population. So what I think is really important and what I really want to see the fields moving towards is always providing the rate of alloimmunization per unit transfused.

Because for instance, **when we just ABO and D match, the rates of alloimmunization in the literature are somewhere between 1.5-3.5 antibodies made per 100 units transfused.** Then when people reported out their rates for C, E, and Kell matching, we found that we cut that by five to 10 times less. So the rate of alloimmunization is somewhere on the order of 0.25 to 0.6 alloantibodies formed per 100 units transfused. And this could be for an institution that might have an alloimmunization prevalence of say, 15% in their chronically transfused

population. But we actually have a much higher prevalence in our chronically transfused population, maybe about 50%, but our alloimmunization rate per 100 units transfused is 0.3.

We know that, just in general, patients with sickle cell disease form alloantibodies at a much, much greater rate than the general population, but probably also, at least from our own experience at a much higher rate than those with thalassemia, for instance, who are often chronically transfused or receive a fair number of episodic or simple transfusions over their lifetime, or for instance, patients with other chronic anemias such as Diamond-Blackfan anemia or patients with myelodysplastic syndrome and even patients with oncologic disorders who require a lot of transfusions over a period of time.

Joe: Got It. So let me ask you this, Stella, and this is me playing devil's advocate for just a second. Why don't we, if we know that patients with sickle cell make these antibodies, that they are at high risk, such high risk for alloimmunization, why don't we just start from the beginning and match them for everything we possibly can right from the start?

Stella: We would love to and maybe one day we will get to at least the most common clinically significant antigens, because with DNA-based assays, we probably can make that pretty high throughput. I think the challenges we have right now are finding enough appropriate donors. So here in Philadelphia, we're really lucky that several decades ago CHOP partnered with the American Red Cross. Particularly, I just want to give a shout out to Dr. Kim Smith-Whitley, who really worked really hard to increase African-American donations because most minority ethnic groups in the US donate less than our general European-based population. So that's true for African Americans, for Asians, for Hispanics. And so, she went out there and sort of grassroots went to the communities to help establish this program to increase African-American donations, because we know that finding the correct matches for C, E, and Kell are much easier amongst African-American donors, and if we were to try to match them, for instance, for the Kidd, and the Duffy, and S ["Big S"] and s ["little s"], with every antigen that we add on to match for, it becomes more difficult to find the number of units.

And when we get to erythrocytapheresis, where we are providing anywhere from two units per session for a pretty small child to up to ten units, sometimes for an adult male, you can imagine finding ten units of blood that say is lacking six antigens can be quite challenging. So I think increasing African-American donations throughout the US is really important. But then also we probably need to find a way to make it a little bit more "high throughput" in terms of testing the donors. So with serologic testing it's really labor-intensive. Now we know that a lot of donor centers are using genotyping or DNA-based assays to predict the antigen phenotypes of donors and patients. And that will probably allow us to get there, I hope, sometime during my career.

Joe: And we are absolutely going to talk about some of the work that you've done to try and figure out how well that can work. So we will get to that when we talk about your really impressive 2018 paper.

But before we get there, Stella, I wonder if we can just go back in time a little bit. You were mentioning that that the traditional ways that, that people have used to try and prevent alloimmunization and sickle cell included, of course, judicious use of blood in these patients, phenotype-matched red cells, at least for C, E, and K, as you mentioned before, and increasing minority donations.

That was kind of the landscape onto which you dropped your 2013 paper in *Blood*, that I really have...as I said, I've mentioned before that I've heard about this paper for so long and I'm so glad to talk to you about it. It was called "High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors." And I think this paper really opened a lot of eyes, Stella. It certainly opened mine. And I wonder if you would just kinda take us through what you guys were thinking when you decided to do this paper, what was the impetus for it, and just the basics of the paper and let's talk about that as we go through.

Stella: Sure. So basically here we had been transfusing patients with this "Blue Tag Program" of African-American donors for almost, I would say 15 years pretty consistently, because the program had taken a few years to grow at first. In general, I would say that if you ask our hematologists, and especially those that proceeded me, what was noticed was that we used to have a lot more patients who, as we said before, were "untransfusible." So I remember in my residency here at CHOP that we had patients who would come in and it was a really big deal to transfuse them because they would get steroids and all this pretreatment because she really needed to be transfused. And that really stuck in my mind. But most of the time she could get transfused when necessary. Basically, nobody had really looked at our alloimmunization rates after this 15-year period of transfusing patients with Rh-matched minority donations.

And so basically coming out of fellowship, I took it on as a project. What I first noticed was, "Hey, this patient's E-negative and they made an anti-E," or "This patient's C-negative and they made an anti-C." And I, at first, was like, "I think we're not getting the right blood!" I was really alarmed, and I brought this to the attention of the people in the blood bank and to our sickle cell program, and basically when we went through carefully and looked at the whole cohort, we found that Rh antibodies, or antibodies directed against the Rh system, were actually the most common antibody that we saw. So **nearly two-thirds of the antibodies formed in patients who were transfused with Rh-matched blood from minority donors were making anti-Rh antibodies.**

Joe: I have to interrupt you Stella, because that is such a mind-blowing thing to me that I think those that are learning blood bank need to hear that again. So again, if you wouldn't mind just say that last part one more time.

Stella: Sure.

Joe: WHAT proportion were Rh in people that you were Rh-matching?

Stella: Two-thirds.

Joe: Wow!

Stella: So two-thirds of our patients who were getting matched for C, E, and K were still becoming alloimmunized to the Rh system, which was very surprising to us. I have to say, on the flip side, we did see that we had probably less cases of alloimmunization to some of the Kidd and the Duffy antigens than probably, if you didn't have African-American units, one would see. So I think there was some benefit in that sense.

I will also say that in our experience we really don't have "untransfusible" patients. We have maybe one right now that we would like to transfuse, but we don't transfuse because he's multiply alloimmunized and has had severe life-threatening, delayed hemolytic transfusion reactions basically every time we try to transfuse him. But I think more importantly for these patients who have made anti-Rh antibodies, what it means is that we have to always make sure that we have time enough to do a full evaluation with their antibodies, their type and screen. And for many of these patients, they're requiring additional antigen-negative blood. And I think the part that is the hardest for me to accept is the patients who are D-positive and make anti-D, and essentially every blood bank will honor that anti-D. So these are D-positive patients now who require D-negative blood.

Joe: I was just going to say that hurts my heart, as a blood banker, with the difficulty with keeping Rh-negative units, as you well know, it's a big challenge.

Stella: Right. So, I had met Connie Westhoff a few years prior and as you know, and many people know, she's sort of the "Queen of Rh." And so we basically started to collaborate, and we started to look at the Rh genotypes of all of these patients and **we found that many, many patients, so almost the majority of the patients, have at least one RH allele that is not what we would consider "conventional."** So for instance, everybody has two *RHD* alleles (unless you have a deletion of the *RHD* allele) and two *RHCE* alleles. We found that **amongst our patient population, 87% of patients had at least one allele that was altered**, which is super-surprising, because in all other populations, and particularly if we compare it to the European population, it's less than 2% of the population.

So we went back and then we correlated those that had altered or variant alleles with their alloimmunization history. And so, for instance, say someone is homozygous for the *RHD* allele *DAU4*, then their red blood cells will express only *DAU4*, which is something we consider a "partial RhD antigen," meaning that they're missing some piece of RhD, so one or more epitopes of the conventional RhD. And by missing that, if they get exposed to conventional D antigen (because there they type as D-positive, they can by all means get D-positive blood), they

can become alloimmunized to the D antigen. So we will detect an anti-D in their plasma or serum.

And so, historically, if you look back at some of the literature of alloimmunization in patients with sickle cell disease, what many had considered autoantibody... So a D-positive patient making anti-D, or a e-positive patient who made anti-e, those are always thought to be autoantibodies. But now we know that some of those patients or most of those patients, probably that antibody was not an autoantibody, but actually an *allo*antibody because they lack some epitope of the conventional Rh antigen to which they have become immunized to.

Joe: Totally get it. I want to make sure that we ask the elephant in the room, "So what?" question, and that's this: So you found that even with these patients who were getting matched blood primarily from minority donors, that they were still making antibodies to a significant extent. So what I think is important for us to understand, and you mentioned this in the 2013 paper, you've described it more extensively in an early view paper in 2019 that will be linked as well, the "so what?" question, which is, "Okay, so they make antibodies. Why do we care so much? What's the big deal for making antibodies? **Is this actually causing problems for these patients?**"

Stella: So the answer is "**Yes.**" So what we did was we looked at the patients who had made antibodies and basically went back to their medical records from when they had made the antibody. So the patient made, for instance, anti-e in 2005, and looked at their other laboratory parameters, because that's something that's not subjective. It's not like, "Oh, they had more scleral icterus at the time, or they felt more tired at the time." Particularly for patients who are chronically transfused, our practice is to always get a CBC and a hemoglobin quantification prior to the transfusion, whether it's simple transfusion or by red cell exchange. And basically the goal is for most of these patients, to maintain their hemoglobin S less than 30%, but for some it's less than 50%. Those are usually the two numbers that we are making our basically highest hemoglobin S that we want to see prior to transfusion.

And so we went back and we looked at the proceeding 6 to 12 months to the antibody detection, and basically calculated a baseline hemoglobin and a baseline hemoglobin S for each of these patients that had made an antibody and compared their hemoglobin and their hemoglobin S at the time of antibody detection to what we considered a baseline from their last 6 to 12 months of transfusion. And what we used was a pretty stringent cutoff of, they had to be above two standard deviations of their own baseline value of either having a higher hemoglobin S (so if normally they came and say it, 28% was their average, but today they made, we found that anti-e and it was 45%, that was likely going to be two standard deviations away from their average), or if their hemoglobin was two standard deviations below their average when they came in for their transfusion, then we consider that a delayed transfusion reaction.

We were really careful on that paper to not call it a "delayed HEMOLYTIC transfusion reaction" because at the time a lot of people were giving us just feedback on the side that they didn't really feel like we could call it a delayed hemolytic transfusion reaction because patients weren't coming in with dark urine and really very overt symptoms. But I think that over time, I myself have come to the conclusion that for patients with sickle cell disease, where we have this indicator with hemoglobin quants, that if the hemoglobin S is much higher and their hemoglobin A is much lower than what you would expect post-transfusion, what happened to those red cells when they made a new antibody? They've hemolyzed them! So in my mind, that is a hemolytic transfusion reaction.

So for these patients, sometimes it's subtle because they have chronic hemolysis. So these are patients who are used to having slightly darker urine, used to having yellow in their eyes, and used to sometimes not feeling great all the time. So sometimes with delayed hemolytic transfusion reactions, it can be subtle. You might fall over...your hemoglobin might fall over the course of say 5 to 10 days if it's not a very robust reaction. And they're going to make red cells. So if they're dropping their hemoglobin, they are starting to then "retic away." And so what we actually found was that some patients could manage to come back up to their baseline hemoglobin with a much higher retic count than they typically would come in at pretransfusion. But their S level was what was really telling. So we would have patients who typically come in with an S level around 30% but come in in the mid-40's or even as high as 60%.

So I think one of the things for us as clinicians that really makes us pause is the fact that we're trying to transfuse most of these patients who are chronically transfused to prevent stroke. So when you're having patients who are coming in with hemoglobin S's that are not below 30%, we're not reaching our goal. And we also don't know, maybe they didn't come in with an overt stroke, but we know that "silent strokes" in patients with sickle cell disease are really common, continue to occur even in some patients who are chronically transfused. So how do we really know that that patient who had a delayed hemolytic transfusion reaction, DIDN'T have a small or a silent stroke when they dropped their hemoglobin? So all things that would concern us.

Joe: Really, when you analyze this in depth, Stella, in your paper that, as I mentioned, it's right now as of the time of this interview, is an early view in Transfusion from 2019 called "Alloimmunization in patients with sickle cell disease and underrecognition of accompanying delayed hemolytic transfusion reactions." When you looked carefully at that cohort of patients that you were describing, what kind of numbers were you seeing that you could say, "Yeah, we think these patients are hemolyzing?"

Stella: **30%. So nearly one third of new antibodies showed evidence of a delayed hemolytic transfusion reaction.**

Joe: Ouch!

Stella: Which is much higher than what the literature suggests. I think the literature suggests somewhere in the order of 10%, and that's probably the overt ones where patients do present to the emergency room or come back prior to their next scheduled transfusion.

Joe: So I have to ask, because in blood bank world we're accustomed to hearing this particular word that that puts us into great fear, when we hear the word "hyperhemolysis," blood bankers have a knee jerk, "Oh no!" reaction. I'm sure it's not a pleasant thing for you to either say or hear as well. I know it's been called other things. "Hyperhemolysis," "bystander hemolysis," things like that. That is... Well, why don't I let you define that and tell me if you would, after you tell me what it is, were you seeing that as well in these patients?

Stella: So **hyperhemolysis, the classic definition is that they have hemolysis that drops their hemoglobin below their pretransfusion. So suggesting that the patient is not only hemolyzing their transfused donor cells, but actually hemolyzing their own red cells and that's where the term "bystander hemolysis" came in.** So classically for most hematologists in transfusion medicine specialists, you have to have a hemoglobin below your baseline or your pretransfusion value.

I think I've always been very careful to use the word "hyperhemolysis." I tend to just use more "severe hemolysis," because it's really hard to *prove* hyperhemolysis, because the other piece of the equation is that say this is a patient who comes in who normally has a hemoglobin of 6, and then we transfuse them because they're going to have a surgery done and we want to get their hemoglobin to 10 for surgery to prevent postoperative complications. But they make an antibody and then they have a delayed hemolytic transfusion reaction, and when they present to us, their hemoglobin is 5. Sometimes I think that can be hyperhemolysis by what people consider with bystander hemolysis. But I would also argue that that patient went to a hemoglobin of 10, their body saw hemoglobin of 10. And so their kidneys probably stopped making quite as much EPO, and they dropped their retic count, and then when they made the antibody and they hemolyzed all their transfused red cells, the proportion of their own endogenous red cells is going to be lower, because they had decreased their rate of production of their own red cells. And so if it's just below their baseline, sometimes I wonder if it's just because they had shut off their own production of red cells, but I certainly agree that we still do have some patients that probably are hemolyzing their own red cells, because we see it significantly lower than their pretransfusion hemoglobin.

But in our experience we found that there were patients who had severe hemolysis that some people would call "hyperhemolysis" where they dropped their hemoglobin several grams below their pretransfusion hemoglobins. I would say that we saw that more commonly in patients who had come in for transfusion for an acute complication or preoperatively rather than those that had come in for their routine chronic transfusion visit.

Joe: Well so that brings us to an interesting place, Stella. I mean, we've kind of taken your papers out of order slightly, but I think the thought processes flow pretty well. In other words, you've pretty clearly demonstrated not only that even if you match sickle cell disease patients as perfectly as we can, or at least, by standard of care, even with minority donors, that there is still a significant proportion of antibodies made. As you mentioned, due to the...presumably due to the genetic variations that you already described. You've described the fact, not only in your 2013 paper, but in this new paper that there are pretty significant consequences of this happening, 30% of delayed hemolytic reactions, in these antibodies along with some that could be considered by some severe or hyperhemolysis.

So that kind of brings us to, I think, where we were when you published your paper in late 2018 that again, everyone, will be linked on the show page, "Rh genotype matching for transfusion support in sickle cell disease." And correct me if I'm wrong, Stella, but this seems like this is at least the beginnings of you and your group's attempt to try and say, "Well, where do we go from here? What can we do to make this better?" Is that an accurate way to describe what you guys were trying to do?

Stella: Exactly. I would just back up in time a little bit, and I would say that one of the perplexing things to us was that in our 2013 paper, when we published that, we couldn't explain all the antibodies. So, if we looked at the patient's Rh genotype and correlated it with their antibodies, we saw some patients who only had partial antigens and then made the antibody to that antigen. So for instance, they're e-positive. They only have partial e-positive red cells, and they made an anti-e. That actually only explains about one-third of the cases.

So two-thirds of the cases were situations where we either had a patient, for instance, who is D-positive, could have conventional *RHD* alleles (or had one conventional and one altered), and made anti-D. A by everything we know about immunology, that seems wrong, right? Because if the patient makes the conventional D antigen, they should be protected against anti-D. And then, as I mentioned before, we saw the patients who are E-negative or C-negative and made antibodies against those antigens. And sort of prospectively then, we started catching some of those. And what we did here was we went back, because we save the segments of transfused units for a couple of months. And when we had that C-negative patient who made anti-C, we actually went back to the last three transfusions and re-typed all the blood ourselves, and found that they got all C-negative blood. So we were perplexed. We have patients who have conventional alleles making the antibody or we have patients who are antigen-negative making the antibody to an antigen that we were giving them negative blood for. And sometimes these were associated with hemolytic transfusion reactions. So we were seeing that elevated S on their next transfusion visit.

So that actually brought us to **looking at the donors** more carefully. So there's no suggestion that Rh variation has anything to do with sickle cell disease. It sort of was kind of known that probably it's just the very genetic heterogeneity that we see in Rh is probably just likely because of the very "old" population that we have

in Africans versus the European population, which is much newer. So we went and we looked at African-American donors. So we didn't handpick them. We basically took 600 consecutive donors and genotyped them. And what we expected, and what we found, was that they had the same heterogeneity at the *RH* locus as our patients. So pretty much almost all the alleles lined up in the exact same frequency as patients with sickle cell disease who are of African descent.

And so that said to us (or we then hypothesized) that some of the antibodies that we're seeing are because patients are being exposed to a different variant than their own variant, or they can be conventional and be exposed to another variant, and making antibodies. And to confuse things more, we have these things called "mimicking antibodies" where basically we think that some donor units that have a variant D antigen can actually elicit an immune response in a patient that makes it look like they made an anti-C. So even more confusing.

Joe: [Laughs] That's messed up, Stella!

Stella: Well that's the limitations of our testing by serology, which, not to say anything bad about serology, because serology is fast and inexpensive and helps us every day. But so basically what that brought us to say was, "Well, you know, we are in the era of personalized medicine, are we not? And so, why not personalize it for patients with sickle cell disease who require chronic transfusion, which basically prevents stroke and many other complications that are life-threatening?" And we asked whether it'd be feasible to genotype-match patients. So whether we can look at a patient's genotype and then provide them with red cells from donors who would be genotypically compatible.

And, what we did to answer that question was we sort of took real life data. We went back to our transfusion records for four years and basically said, "Patient X was transfused on this day, required this many units," and so on and so forth. Because basically one patient with a similar genotype could potentially be competing with another patient with a similar genotype for donor units. And we did, with the help of some very savvy bioinformaticians, did a virtual matching, where essentially we basically made a "virtual blood bank" with African-American units and with Caucasian units, and took the frequency of alleles amongst those two populations and built a blood bank inventory, essentially. And then basically every day over four years looked to see if we could find the matches for the patients that we needed.

And just to put it in perspective, we approximately issue about 30 units of blood per day from our blood bank for patients with sickle cell disease Monday through Friday, a few on the weekends. And over the course of a year, over the four years that we looked at, we were issuing between 6,000 and 7,000 units of blood for patients with sickle cell disease, encompassing approximately 200 patients per year that had required a transfusion or were chronically transfused. And **what we found was that if we were to serologically match them with CEK-matched units from African American donors OR genotype-match them, if we were to give them sort of a "perfect genotype match" where we match them with**

only alleles that they had, it would take about two times more donors per day recruited to provide genotypically-matched cells to these patients.

So the bottom line was that from a numbers game, if you have a blood bank that already provides a significant number of your units from minority donations, we think it's feasible. I think that the main challenges now are the cost of *RH* genotyping and then also the management of having all the donor data, having all the patient data and is having a system that crosstalks between hospitals and blood suppliers in terms of phenotype matching.

Joe: And I wanted to ask you a little bit about that, Stella, because one of the things that learners in blood banking are, I think, still trying to get straight is, when we look in some hospital transfusion services, more in traditional immunohematology reference labs (and I'm not talking about like Connie's lab, like I consider Connie's lab to be a super lab), but for the rest of us *normal* people (and that's no offense to Connie, you know, I LOVE Connie!), but for the rest of us, we have...the technology that's available for us for *RH* genotyping is...I would describe it as fairly simple. I mean there's a couple of systems out there. I guess what I wanted to ask you is, are we talking, when you're talking about "matching," we're not talking about just the simple genetic testing, molecular testing that's done in a lot of reference labs. We're talking about, for example, high-resolution genotyping. Is that correct?

Stella: Right. So currently, high resolution *RH* genotyping is only done at specific immunogenetics labs like Connie's, like the Blood Center of Wisconsin, or other large reference labs such as the one in Philadelphia, which is affiliated with the American Red Cross. There's only a handful of labs that really do the type of *RH* genotyping you would want for your patient with sickle cell disease. So many people are now familiar with the genotyping platform that recently was FDA-approved, which is the human erythrocyte antigen chip. And while that does pick up a few variants that we see in patients with sickle cell disease or African American donors, it's not comprehensive *RH* genotyping.

And so, for comprehensive *RH* genotyping, it requires typically many different assays being run. So it's not even a hundred percent comprehensive, but it's, you know, 97-98% comprehensive for patients with sickle cell disease. And that cost is prohibitive for testing donors right now. I think in the future, with certain next generation sequencing technologies that have to be adjusted for *RH*, because *RH* is a duplicated gene family. So *RHD* and *RHCE* are very, very homologous. And so, for most next generation sequencing technologies that sequence relatively short reads, basically the way it's analyzed will be confused with *RHCE* and *RHD*. Basically it'll map sequences that really belongs to *CE* on *D*, and vice versa. Especially because in Africans, the variants we see were due to hybrid genes that arose where parts of *D* went into *CE* and vice versa. So you can see how technology has to be a little bit more advanced before we can get to NGS-based assays, which would bring the cost probably down to a level where we could do that for donors.

Joe: It seems like the future has potential to be very exciting for us and hopefully to make some great improvements on everything that we're doing. But for now, I guess Stella, what is your current thought on what "state of the art" transfusion for these patients should be? Where should, you know, "Blood bank X" that's sitting in, you know, in a place that's not necessarily a massive sickle cell referral center....They have a patient that comes in with sickle cell. What are the things that they should be thinking about in terms of priorities for these patients?

Stella: Sure, that's a great question and I get asked that a lot. So I think we're not at a point where we can provide "perfect" transfusion care for patients with sickle cell disease, but **what we suggest is, if feasible, to get the *RH* genotype and an extended red cell genotype on all patients with sickle cell disease.** And the reason for that is that when you do have the patient who comes in with a new positive antibody screen, having that information really helps you more quickly determine what your antibody specificity is. So for instance, because our policy had always been to at least have a red cell antigen serologic phenotype, where we have about a dozen and a half antigens that are typed and we know the patient's status, with the genotyping, we now know the status of 35 antigens, and sometimes those are the antigens that we do see antibodies made by patients with sickle cell disease. So things that we don't typically type for. So, one, that helps you determine their antibody specificity more quickly, but it also sometimes helps us when we see a patient with particular *RH* alleles that have, for instance, homozygous partial e alleles. When we see that anti-e, we're not super-surprised and we don't keep on working it up and saying like, "Is it really an anti-e?" It basically says like, "This patient had a partial, they were at risk, we knew they were at risk, we couldn't match them for their partial e right now. So they made an anti-e." So, and that in turn allows us to issue that blood much quicker than if we're still trying to perform the evaluation. And for us and sometimes other blood banks, that evaluation doesn't always happen in-house. It happens at our reference lab, which typically takes 24 to 48 hours, especially if it's not too complicated. And as you know, when people order blood, they want the blood now, not like 48 hours from now!

Joe: Amazing how that works! Yes!

Stella: Right! So that's one thing that we think helps. And the other thing that what we've implemented, and I know other institutions who care for a fair number of patients with sickle cell disease have also implemented, is that there's this one allele, it's called... It's a hybrid allele where basically a piece of *RHCE* went into *D*, and that hybrid allele causes no expression of any *D* antigen, even though it's actually your *D* gene, but it causes expression of a partial *C* antigen. And these patients type strongly positive for *C*. So they typically, when they're matched for blood, they can get *C*-positive blood. And what one group in France has shown is that 30% of these patients then go on to make anti-*C*, because they're exposed to conventional *C* typically.

So for patients that are *C*-positive, but have this hybrid allele but do not have another *RHCE* allele that expresses the conventional *C*, we actually put them

prophylactically on C-negative blood...which is consistent with our policy of providing C-negative patients with C-negative blood. We know this patient population with this partial C is at very high risk of making anti-C. So we put them on prophylactic C-negative blood.

There's a few other cases where if someone's highly alloimmunized, we might by their genotype, provide them with antigen-negative blood, but that's more an individual case-by-case basis. So basically I think that having that genotype up front helps you with your antibody identification, and for patients with the C due to a partial C, providing prophylactic C-negative blood can help prevent an anti-C from forming.

Aside from that, I think that all patients with sickle cell disease should get Rh and Kell-matched blood, which is surprisingly not always the standard of care throughout the US, and part of the reason why we say that is because we know that patients who make one antibody are then at risk for forming additional antibodies. So sometimes people think, "Well, have them demonstrate that they're a responder," they say, "that they're a patient with sickle cell disease that *will* make antibodies. Then we'll start antigen-matching you." But I think for these patients who might require transfusion in an emergency scenario, we want to prevent all antibodies.

Joe: I'm right there with you. Completely agree on that. Well, Stella, this has been an amazing experience for me. It's been wonderful to talk to you to, to hear your expertise on this, which is tremendous, and I think you've given us a lot of great things to think about and hopefully some really good thoughts moving forward that will help us work with clinicians, us in the blood bank, work with clinicians to make care of patients with sickle cell disease better. So thank you so much for taking the time.

Stella: Thank you for having me.

Joe: Hey everybody, it's Joe with just a few closing thoughts. My thanks again to Dr. Stella Chou for her really deep thoughts, sometimes, on sickle cell disease. I have to say, you probably figured this out from the episode, but Stella "swims in the deep end of the pool," as we like to say! She is brilliant, and I'm really grateful that she was able to share that information in a way that I hope is really, really useful for you.

I would also like to thank my new assistant editor of Blood Bank Guy, Dr. Daniela Hermelin [NOTE: Follow Daniela on Twitter, [@HermelinDaniela](#); you won't regret it!], who was just a MASSIVE help in preparing the continuing education materials for this podcast.

And speaking of those, remember that if you are a physician or laboratorian, you can go to www.wileyhealthlearning.com/transfusionnews, get your hour



of totally free continuing education credit. While you're there you can find a lot of other episodes, a lot of other things that you can listen to and get equally free continuing education episodes, including those from 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/070. I would highly recommend that you go there. You can find links to all three articles that Stella and I discussed in this episode, and I think they'll be really very, very useful for you.

You've heard me say this before, but again, if you can go to Apple Podcasts and give this podcast a rating and review, it can really help get it in front of more people, which is again I'm really trying to do. Thank you so much for those of you who have already done so.

I have lots of fun stuff coming up in the coming weeks; an episode on the brand-spanking new American Society for Apheresis guidelines for therapeutic apheresis, as well as a great episode on neonatal platelet transfusion with Dr. Martha Sola-Visner. I'm really excited for you to hear both of those. But until those days come, my friends, as always, 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.