

**BBGuy Essentials 057:
Nonspecific Antibodies with Brenda Grossman
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Joe Chaffin: Hi everyone! I am very happy to welcome you to Blood Bank Guy Essentials, the podcast designed to help *you* learn the essentials of Transfusion Medicine. This is Episode 57 and my name is Joe Chaffin. Today, I'm interviewing Dr. Brenda Grossman about an all-too-common challenge in Transfusion Medicine, what she calls "Antibodies of Undetermined Specificity." You're really going to want to hear this one.

First, you should be aware: This is NOT a continuing education episode. You can find other episodes where physicians and laboratorians can earn those continuing education credits for absolutely no charge whatsoever at bbguy.org/podcast. You just look for episodes that end with the letters "CE." You can also visit wileyhealthlearning.com/transfusionnews. The continuing education episodes there are brought to you by transfusionnews.com, and Transfusion News is brought to you by Bio-Rad.

So, back to today's episode. One of the more frustrating things we deal with in Transfusion Medicine, I think, is the situation where a person is being screened for unexpected red cell antibodies (we typically call that an "antibody detection test" or more commonly, an "antibody screen"), and everything looks good except for *one little reaction!* One little thing suggests an antibody might be there that could cause a problem, but it's not specific. You go on, you work up the case even more specifically, or in even more detail, and you can't say exactly what it is, but you can't deny that it's there! This situation really frustrates everyone, and blood bankers everywhere have sent me emails asking me, "What do I do in this situation?"

My guest today is Dr. Brenda Grossman, and Brenda knows your pain! She is a professor of Pathology, Immunology, and Medicine at Washington University in St. Louis, where she is medical director of Transfusion Medicine Services at Barnes-Jewish Hospital, as well as director of the Clinical Pathology Residency Program and the Transfusion Medicine Fellowship Program (just for good measure). A few years ago, Brenda and one of her residents (and later colleagues), Dr. Chang Liu, decided to do more than just *wonder* about these antibodies. They put together a study to analyze what actually *happens* to patients after they have one of these nonspecific antibodies. What do we find when we look closer? She's going to describe all of that for you today.

You should know, I interviewed Dr. Grossman before I realized that her co-author, Dr. Liu, was going to be doing an AABB eCast on this same topic. He did that eCast on September 12, 2018, and it was really great! I encourage you to listen to the recording of that webinar. However, his focus and my focus are a little bit different. My interview with Brenda is a

little less “technical” than what Chang presented, and I think you will find it of great value.

You should know, you can get a link to the paper we are discussing, which is called, “Antibody of undetermined specificity: Frequency, laboratory features, and natural history,” in the May 2013 journal Transfusion, on the show page for this episode at BBGuy.org/057.

So, here’s my interview with Dr. Brenda Grossman on antibodies of undetermined specificity:

Joe: Well, hey Brenda, welcome to the Blood Bank Guy Essentials Podcast.

Brenda: Thanks for having me. It's an honor.

Joe: Well, it's my honor, my honor! Absolutely. I'm really excited that you're here today, because I think that this topic that we're going to describe has so much practical meaning for those of us that work in blood banking. And I would say up front, for those of you that are listening, no matter what you do, no matter how you interact with the blood bank, this topic is something that will probably impact you. Certainly some more than others. But today we're going to talk about what Brenda calls "Antibodies of undetermined specificity." And Brenda, it's a great topic. It's, as I said, a super-practical topic. I wonder if before we get into the details, if you could just tell me a little bit about how you got interested in this, how did this become something that was on your radar?

Brenda: So, like many of the projects that I do here, it started on rounds one day when an astute resident who later became my fellow and my co-author on this paper ask the question, "What are these nonspecific antibodies of undetermined specificity reactions that we're seeing?" And I started my response with, "Well, some of them they are probably to low frequency antigens, some of them are probably antibodies that are evolving or 'evanescent,' and some of them are probably just agglutination nonspecifically." And he said, "Well, how do you know that?" At that point I just didn't feel it was right to say, "I've seen 'em, you know, I just recognize the pattern!" And so I said, "You know what, why don't we do a study and retrospectively look at these and determine what they are, what the natural history of these are, how often are they occurring?" Because he was right, we were seeing many, many more of these reactions as we continued to do testing.

Joe: Well, Brenda, I'm really glad that you guys did have curiosity about that because it resulted in what I would tell you is, is really kind of one of my favorite papers and that may say something about how much of a nerd with immunohematology I am, but I love this paper! And it came out in the May 2013 journal "Transfusion," you and your co author, Dr Chang Liu, put

out this paper which you titled, "Antibody of undetermined specificity: Frequency, laboratory features, and natural history." And again, that's May, 2013 Transfusion. Everyone, this will be on the [show page for this episode on the Blood Bank Guy website](#). So please check that out there. But Brenda, before we go into what you guys found, I wonder if we could talk about...Well, let's step back: For those who aren't necessarily familiar with everything that we do in blood banking, in a perfect world, how does normally the flow go? How does the process go to determine one of these antibodies? We talk about this all the time: "We sent a sample to the blood bank. The blood bank found antibodies." What does that mean? What have we done to get to that point?

Brenda: Okay, so let's even step back one more step. Why are we doing this to start off with? And we're doing this in order to detect and identify clinically significant antibodies so that we can find compatible blood for the patient in need. So we receive a tube in the blood bank that has been properly labeled, and then we first do what's called a "type and screen," in order to determine the ABO type, the Rh type. And then we take the sample and we take the patient's plasma, and we mix it with reagent red cells that have a compliment of antigens on their surface. The FDA has specific red cell antigens to which we need to determine if there are antibodies in the patient's plasma. And so, we mix them and through various washing and incubation and there are different methodologies, so depending on what we're doing in the end, we either see agglutination as the end point in a positive reaction and no agglutination in a negative reaction.

From there, depending on what the results of that is, if we have a positive antibody screen, then we will go on to try and identify which antibodies are in the patient's plasma. And we do that by taking a larger set of cells; it's usually 10 or 11 cells on a panel, which have various antigens on their surfaces. And again, we mix, incubate, and then read the reactions. And then based on the reactivity profile, we can determine whether a specific antibody exists. In a perfect world, we can determine what that specific antibody is (if such reactions occur). But sometimes, we will have ruled out or been able to rule out all clinically significant antibodies in which the FDA requires us to do. Sometimes we're left with an extra reaction, which we don't necessarily know what it means! It's there, it's clearly reactive, but we have ruled out antibodies to every antigen on that cell that is on the panel. So from there is where people diverge in what they do. And so the perfect example is, we do an antibody screen which is two or three cells. And there is a positive reaction. We go on to do a larger panel with 10 to 11 cells, and they're all negative. Well, based on that panel, we probably can rule out everything, but we still have this reaction. And so that's where we would call this an "antibody of undetermined specificity." At this point, I'm not sure what that is.

Joe: And that's the problem, isn't it? We've got a result that we don't know exactly what to do with. So I think that's a great background and

introduction for us. If you want to know a lot of technical details about everything that, that Brenda just talked about, I did a two-episode greatly detailed podcast with Sue Johnson. You can find that at BBGuy.org/050 and BBGuy.org/051. So you can find that there. So we're not going to get into the nitty gritty of all this, but I wonder, Brenda, if you would just outline kind of the main options in terms of testing platforms for how we do that antibody screen and antibody identification testing that you were describing.

Brenda: So there are several choices. We could use "gel technology," it's microcolumn, gel technology, "solid phase," and "tube testing." Tube testing is what we have been doing for years and years. In the 1990's, we started using solid phase and gel technology, and that's when I think antibodies of undetermined specificity became a real entity, because with gel technology and solid phase, these technologies are more sensitive, and so they are picking up reactions which we may have missed in the past in tube testing. And with that increased sensitivity, we're also losing specificity. And so now, we are seeing these reactions more commonly, and they are becoming a nuisance! Some of them are truly just nothing BUT a nuisance. And the problem is that we have to treat them with respect because they may actually be clinically significant antibodies that, as I said, are developing or "evanescing." And so, this increased sensitivity is GOOD, but it also increases our workload, and it increases the time to release compatible blood to the patient, which is a problem.

Joe: It's the classic "double-edged sword," right? Everybody loves the sensitivity and that's awesome, but there's another side to it and that's exactly what you were describing. So why don't we, if you don't mind, do you have a case example that just kind of illustrates for people how something like this might occur in the real world?

Brenda: Sure. So let's say we had a 96-year-old female who had a history of aortic stenosis who presented for aortic valve replacement. We actually had history on her from a previous admission, at which time she had a negative pretransfusion workup, but she received a red cell at that admission. On this presentation, we did a type and screen and we had one cell that was positive on her antibody screen, then we did gel antibody identification and every cell was negative and we were able to rule out all clinically significant FDA-required antibodies. And so here we were left with a person who we're trying to get ready to surgery and we didn't know whether this unexplained reaction was a low frequency antigen or a developing antibody. And so we were pretty much "forced," at least in my mind, to do an antiglobulin crossmatch, because even though the AABB Standards says you have to rule out all clinically significant antibodies, even though I had done that, I still wasn't that SURE. So we went ahead and did an antiglobulin crossmatch, and at that point I felt very comfortable giving this woman compatible blood. If I had not had that reaction, I could have basically done a "electronic crossmatch" and saved a lot of time

getting blood for her. So that's the usual case. Sometimes it's one reaction, sometimes it's two or three. It's rarely many extra reactions.

Joe: All right, Brenda. So you guys had this great idea to take an actual look at what really happens to people in that situation where they have a reaction that doesn't really correlate with any specific antibody, and, as you said, in your case, you gave the patient crossmatch-compatible blood and followed through. So what I'm wondering is, it seems like so obvious that you know, this would be something that would be important. Had anybody really studied this formally before you guys?

Brenda: So the best of my knowledge, no! It was always said in studies that were looking at the sensitivity and specificity of these new technologies that there were these unexplained reactions, but I don't think anybody looked at frequency, the laboratory features, or what happened to these. I mean they were obviously known to occur...

Joe: I would agree by the way, because I have lost many hairs pulling them out over these things over the course of my career too.

Brenda: I think we all have!

Joe: For sure. Okay. Well, again, one last little thing before we get to the details of your study, and it seems like a minor thing, but I want to give you the chance to address it. Going along with your study in that May, 2013 Transfusion, there was a really nice editorial written by Chris Tormey and Jeannie Hendrickson that kind of commented on your paper, but I was interested in the fact that you called it "antibody of undetermined specificity" and in that editorial, it was designated as "antibody of undetermined significance." And my first thought was, "Well, no big deal," but I just want to know from you, is there a difference there? Is that...I mean, I know there's no formal, defined, absolutely "this is what we call it," but do you think there's any significance in the difference between those two words? (No pun intended)

Brenda: So, sorta! As you say, I think we all call these reactions something different. Some people call it as I did, and I think "undetermined significance" is a valid term, but I called it "undetermined specificity" because I don't know what the specificity is. I don't know whether it's clinically significant or not significant. It may not be *anything*. I'm having a hard time defending what I called it versus what they called it, but I think the word "significance" and "specificity" are a little different. So, you know, in the end it's all semantics, and you're right, it took me two times reading their articles before I even realized that we called it something different.

Joe: Well I don't want to keep everybody waiting anymore. Brenda, we need to...let's get to your study. So, I wonder if you would describe your study, which I understand was kind of done in two different parts or two different

ways of looking at looking at this whole thing. So I'll just throw it to you. How did you guys approach this?

Brenda: So the first thing we did is, we needed to determine the frequency and look at the laboratory features. And so, we had a 30 month period, from July 2009 to December 2011, where we retrospectively reviewed any chart that had a positive antibody screen. Then we determined what that antibody was. So, for those that we determined were antibodies of undetermined specificity, we collect certain variables. But let me first define what we call “antibodies of undetermined specificity.” We use this term at our institution to report unexplained reactions when antibodies against the FDA-specific red cell antigens have been ruled out (and this is codified in 21CFR section 660.33 for those of you interested). So the variables that we looked at were gender, age, type and screen results, antibodies that were concurrent with this AUS, the number of positive reactions that were attributable to the AUS, the strength, the results of backup testing when we used alternate methods, whether they had previous antibody history, whether their auto control or DAT was positive, and whether the AUS persisted or disappeared, with new antibodies identified in subsequent workups. Then, the second part, we took a subset of AUS patient samples and examined the laboratory features of those AUS to determine what did they look like. And then, we took a subset of those that we followed prospectively to determine what did those things look like later on, meaning in subsequent antibody screens?

Joe: And just to be clear, Brenda, this was all done at your hospital. Just it was a one hospital...

Brenda: ...it was a one hospital. Yes.

Joe: So make sure I understand: Part one, mostly just to determine how often it occurs. Part two, to analyze the variables a little more and determine what happens to at least some of these patients who have these antibodies form. Is that a fair way to put it?

Brenda: Correct.

Joe: So tell us a little bit about your hospital, just so we know the background against which the study was occurring.

Brenda: So, we are over a 1000 bed hospital. We have about 90,000 ER visits, 40,000 units of red cells (40 to 50 depending on when you measure red cells transfused), and about 40,000 surgeries done a year. It's a big hospital. And so we honestly have decreased our red cell transfusion tremendously over the last few years with patient blood management programs. So we're down, we're close to 45,000, down from a high of 60,000 red cells transfused.

- Joe:** Wow! Impressive! Okay. And so during that first period when you were just looking for frequency, how many, you tell me, how many patients did you guys look at over that timeframe?
- Brenda:** So, we had a little over 135,000 patients undergo pretransfusion testing during that period, and of those, about 4.5% of the patients had a positive antibody screen. And so that ends up being around 8,000 antibodies detected in about 6,000 patients.
- Joe:** So what did you find of those 8,000 plus antibodies detected?
- Brenda:** Well, the first thing we found out was that the antibody of undetermined specificity was our most common finding! Of those antibodies, 1,400 of them were antibodies of undetermined specificity. We had over 5,000, close to 6,000 were specific alloantibodies. Then we had a few hundred autoantibodies, and a few hundred, or around 400 passive anti-D's, which represents our high-risk OB group. So antibodies of undetermined specificity ended up being our most common finding in a positive antibody screen.
- Joe:** Wow. So more common than any Rh antibody, any Kell antibody, anything like that; an AUS was more common?
- Brenda:** Yes. However, our most common specific antibody was anti-E, and it was close. They both came in at 18%. However, the antibody of undetermined specificity, the decimal point, made it higher.
- Joe:** Gotcha. Just by a nose, AUS won. And I use "won" in quotation marks because that I'm sure it doesn't feel to you and your transfusion service, like that's a win.
- Brenda:** No, not at all! We know what to do with an anti-E.
- Joe:** Right. Okay. So I mean, just to put that into perspective, I mean, we can all do math, but that's close to one out of five antibodies identified is an AUS in that setting? It's no wonder I'm pulling my hair out, Brenda!
- Brenda:** Yes, yes, yes! And again, as I said, you know, if we're going on this path and we think we have an anti-E, we know exactly which cells to pull, and how to prove and disprove, but if we don't really have any idea what this could be, we truly have to continue on this pathway to identification, which may mean just more panels.
- Joe:** Well, I'll just throw in my perspective as someone who's right now the medical director of a reference lab. Oftentimes in reference lab world, we see these from hospital transfusion services. Now, yours is obviously, you're a big place, and I'm sure your techs are very skilled and sophisticated, and I'm sure you guys are capable of a lot. In smaller places, oftentimes we in reference labs get these, and we have the same

question: How far do we go to try and get to the point where you say, "Yeah, that's enough. It's an AUS" or whatever the local language is. They can be really frustrating. I'm sorry to persevere on that, but I just want people to understand that this is a big resource-user in many ways. It takes a lot of time to get to the point where you kind of go, "That's as far as I can go."

Brenda: Exactly. And we all do it differently. That's another issue here is that there is no standardization as to how you are supposed to resolve these, or if you need to resolve.

Joe: So, we know that we've got this, as you said, the number one, by a nose, most common single finding was, was one of these "antibodies of undetermined specificity," as you call them. So I wonder what sort of analysis were you able to do, or was there anything that you could learn from those before we get to the second part where you followed some patients to see what happened?

Brenda: The first big group was basically just frequency. It was a subset of 174 patients, which we looked at the demographics. We had a two to one ratio of women to men, which we would expect because, they are not only exposed to alloantigens in transfusion, but also with pregnancy. Our mean age was 55, which may reflect the hospital as opposed to anything else. We looked at whether they had previous antibodies or not, and the majority of them, over 50%, had never had another antibody, and never had a history of a previous AUS. There were several patients that had concurrent antibodies identified, but the majority did not. And more than not, they had a negative autocontrol, so there were no autoantibodies detected at the same time.

Joe: I'm not sure how much that helps us, but I mean it's...

Brenda: It was purely descriptive.

Joe: Fair enough. Fair enough. I see. Okay. Well, was there any theme that popped through in terms of the strength of the reactions, or the number of positive reactions that you found in these patients?

Brenda: The majority of these had **weak to 1+ reactions, and if you look at the number of cells that were positive, the majority were one to two.** My coauthor made a fancy three-dimensional graph that you can look at it in the paper, but the important point is that if you look to the left, that's on the x axis, that's the number of positive reactions, and it's really one or two that pop up. You can, you will be able to see, if you look at the article, that there are very few of these antibodies of undetermined specificity that react with multiple cells.

Joe: I will tell you, this is completely anecdotal, but my perspective again, as a reference lab director is that when I see these, and I think most people

would say this, that's the kind of thing that I expect. It's pretty uncommon for me to see a single 4+, super-strong unexplained reaction. It happens, but I haven't seen it very often. Obviously you guys, in your paper, saw a few of them but not a whole lot.

- Brenda:** Yes, and I would really be very concerned about that, and I would assume that that was something significant. And that is the one that I would pull the panel cells out and look at it very hard to see what antigens that it had on it. And I would, even though I probably would have met the criteria for ruling out an antibody, specifically antibodies to the Kidd group, I probably would do a few more cells just to make sure.
- Joe:** Absolutely. Okay. Well, so I think we can say that we certainly learned something from the first part of your study. You know, several interesting facts, but I think a lot of the questions that we have, at least we can start answering with the second part of your study, which is where you analyzed going forward, a group of patients that presented with these antibodies of undetermined specificity. So I'll again, I'll throw the floor back to you, Brenda. What did you guys find with that group?
- Brenda:** So we had 45 patients who had an AUS for the first time and had at least one follow-up workup later. And of those 31 patients, or 69%, the AUS persisted for at least our median follow-up of eight days, with a range of 2-60 days. 14 of those patients, the AUS disappeared. But of those 14 patients, 7 of them developed a specific new antibody. Yes, that's what we're concerned about. 1 was an autoantibody, 9 of them were alloantibodies. Our median follow-up was 8 days. And so we probably are underreporting because we didn't have longer follow-up. And as we blood bankers know, delayed serologic reactions occur at a much higher incidence than delayed hemolytic transfusion reactions. And therefore these patients may have not come back because they had no symptoms, and we don't have a follow-up. So, this is about 15%.
- Joe:** Brenda, forgive me for interrupting. What kind of antibodies are we talking about? Are we talking about just boring benign things? Are we talking about significant things?
- Brenda:** We're talking about significant things. Anti-E's...mainly anti-E's. The majority are the Rh group, and that's something that other people have found also.
- Joe:** So, as I look at the chart in your paper, I see a couple of...I see, you're right, mostly Rh antibodies. I see a couple of Kidds.
- Brenda:** And -s.
- Joe:** ...and a little s. Those make me worried as well. Obviously the Kidds especially. I have to say, those are somewhat startling findings, that we go from a scenario where you've got weird isolated reactions that you're like,

"Eh, I don't know what to do with them," to all of a sudden, a median of eight days later, we've got major antibodies hanging around that I think that should make everybody sit up and take notice.

Brenda: You know, after we presented this paper, there were several people who approached me and said, "Well, **what do you do?**" And so what we decided to do was, we treat these as clinically significant antibodies. And so **all patients who have antibody of undetermined specificity in their record, we treat them as if they have a clinically significant antibody and we do an antiglobulin crossmatch** on them, right now, for the rest of their lives, because we're not sure if we didn't miss. Now other people have taken other approaches. Other people have, at the time of the antibody of undetermined specificity, they have chosen to work them up further. At the University of Alabama, they published an article in "Laboratory Medicine" where they chose to treat their specimens that had resulted in antibody of undetermined specificity, they treated them with ficin enzyme treatment, and were able to identify a significant number of new antibodies. In fact, they had about 20 new antibodies that they were able to pick up. Again, most of them were Rh antibodies, but there were a Jk^a and Lewis antibodies. So they chose to work it up at the time. We basically decided to repeat it over time, and if they are going to develop eventually, they will develop into something and we'll deal with it at the time. When I compare the two, theirs is much more conservative to us, because they are going to prevent, if this is an antibody that's developing, they may prevent giving them incompatible blood, whereas, we're waiting to see what the next antibody screen shows.

Joe: That actually brings up a question for me, Brenda, that I'm going to ask as delicately as I can, but I'm curious, when you saw those patients that had the new antibodies, did you evaluate them to make sure that they weren't hemolyzing or they didn't get incompatible blood, or was that even possible with a group of patients like this?

Brenda: Absolutely! Every time we identify a new antibody, we review it, and we go back and look in their history if we have recently transfused them. And, if we still have segments available...well, first, we look to see whether there's any evidence of hemolysis, and then we look at the patient's history to determine what's going on with the patient. If this is a patient who just had surgery a week ago, and I know I have a new antibody now, I will call the physician and tell them that, because the last thing I want them to do is to think this patient's bleeding and take them back to the OR, when all it was was they were destroying the red cells we gave them last week. Obviously, most of these are not going to be that dramatic clinically. Otherwise we would know what it is.

But when we do get a new antibody, we do go back and look at the history and if the patient is hemolyzing, and it's an antibody...if it's a person who's gotten multiple units, and the antigen is of high frequency, and we have

the segments, we will go back and type those, so that we can give the clinician an idea of what percent of the cells we gave them this person could potentially hemolyze. So, for instance, if it's a new anti-K, I'll tell him, "You know, only 10% of units. So if we gave you 5 units, maybe 1 will be positive." On the other hand, if it's a Jk^a, we know that 75% (or around 75%) of people are Jk^a-positive, whether they're homozygous or heterozygous, and so, that's a huge number. If I gave somebody 5 units of blood, they're going to hemolyze a significant amount. And I want them to be aware of it, because I don't want them to think they're bleeding.

Joe: Brenda, as we move on to the conclusions that you guys had (and I appreciate everything that you've said so far), I wonder if we could just have you summarize again for those that aren't necessarily familiar with everything that we do, when it comes down to it, you guys had some suggestions for potential etiologies, or why these things occur. And you've mentioned some of them already, but I wonder if you would mind just taking us through those again? When you see something like this, I guess in a blood banker's mind, what are the questions that should come up? What COULD this be?

Brenda: Well, is this an antibody against a **low frequency antigen**? Is this an antibody against a **non-red cell antigen, HLA Antigen**? Is this an **antibody developing**, or is this an **antibody that's evanescent**, and for that matter, if they've **previously been transfused**? And then, lastly, it could just be **something agglutinating in whatever method that you're using**. And so those are really the choices. So, at best, it's nothing. And at worst, it's a clinically significant antibody.

Joe: Those are pretty wide variations there. Yes, they are. You know, I have to ask you this because I think it's an important question, and this was raised somewhat in, I mentioned Chris Tormey and Jeannie Hendrickson's editorial, so I'll just flat out ask you this, and I'm saying this a little bit, playing "devil's advocate," I will admit that up front. So, if we have a method that gives us 1 out of 5 (or so) antibody-positive antibody results that don't, at this moment, mean anything, does that mean that's a bad method, Brenda? Is this something that we should say, "Oh wow, this method isn't great, we should consider something else?"

Brenda: Well, you have to raise it against the benefits of the method. You know, gel and solid phase have allowed us to automate our antibody screening. And in addition, with tube testing, you know, the reactions are only readable for a very short period of time. And so, with gel and solid phase, you can come back and look at these reactions the next day. And so for those places that can't have a blood banker there at all times, for the generalist, these instruments are very helpful in allowing blood banking to continue with a generalist running the lab. And again, it allows the blood banker, who may only be there in the day, to come back and look at the reactions and compare it to how it was read out, and hopefully correct any training

that needs to be done if they find something in relatively “real time,” at least before the next night.

Joe: So those of you that are "gel fans" out there, please don't send me nasty emails. I was deliberately asking a question, as I said, playing devil's advocate a little, but I'm certainly on record, Brenda, and I hope you'll agree with what I'm about to say, I'm certainly on record as saying that **there is no perfect platform.**

Brenda: Absolutely.

Joe: You're going to get pros and cons with all of them. Right? Some good things, some things that you wish were different, but in the end, I love the way you said it: You have to balance the things that you get that you don't like against the things that you do like, some of which you've just summarized there perfectly. So, I don't hate gel, everyone. Don't write me emails. That would not be nice.

Brenda: You would be a minority if you did.

Joe: It's true. It's true. Okay. Well, so one other thing I want to talk about before we finish this, Brenda, and it's something that you have already raised. And you had mentioned that your particular facility's approach in cases like this with AUS, is that you do an antiglobulin crossmatch (and again, for those of you that are just learning, that means we're doing everything we can serologically, including an indirect antiglobulin test to ensure that this patient's plasma is compatible with these donor units). So you guys have taken that approach, and other people, as you said, have taken different approaches. So let me ask you again, to clarify why you took the approach that you did? Should we require an antiglobulin crossmatch and patients with AUS?

Brenda: You know, it's my recommendation, because I'm concerned about the “larger than I thought number” that turn into significant antibodies. However, I'm not sure I have the data to be dogmatic in that way. People have done things differently, and some people are more restrictive, or you know, take the workup much further than I would. At this point, we're not doing enzyme treatment, but I think that's a great idea for those that "my gut" tells me (because it's nothing more than my gut) tells me that this may be something. You know, what I was hoping was that we would be able to look at features of these antibodies, or the patient, and determine which one of these had potential. But, it really didn't pan out that way. There really wasn't anything that told me which one of these were going to turn into something.

So, I mean, I think it's really important when you have these, that you look at the clinical context. If I have someone who has an AUS, and they don't seem to get the "bump" that I think they should get when I transfuse them,

and it still continues to be an AUS, that's when I think I need to say, "Well, should I do something else? Should I enzyme-treat this?" If I have someone who has an AUS that sorta seems to hemolyze every time I give them blood, I'm going to look at, you know, what percent of the blood I give them, do they actually hemolyze? And then I'm going to think about the frequencies of all these significant antibodies.

And I have been known to phenotype for Kidd, many people with negative antibody screens. And, you know, my techs, they don't like me, but we found a few that are homozygous for either Jk^a or Jk^b , and when we started giving them negative units that were negative for the antigen they lack, we were pleasantly surprised to find out that they no longer would hemolyze it. So, I really think you have to treat these individually. But at this point, we took it as far as making them perform antiglobulin on all of them, antiglobulin crossmatches on all of them. And then, for those that are particularly worrisome clinically, because like I said, there's nothing in the laboratory features that allows us to determine this, but if there's something clinically, we may pursue it. We always, if we have a screen that's positive and a gel panel that's negative, we'll also do tube with PEG before we conclude that it's truly only that one cell.

Joe: What I'm hearing you describe, Brenda, is really, I guess I would call it the "art of blood banking, the art of medicine." There are lots of decisions that individual facilities have to make, and one person's answers may not work for another facility. So, I...

Brenda: And there's more than one way to do everything.

Joe: Absolutely. Absolutely. I completely agree. So, what we've talked about is, is a scenario that that can be challenging and frustrating, often unsatisfying, but I think what you've demonstrated pretty clearly for us, Brenda, is that, you know, in this population that you guys looked at, there is a chance that this could be something significant. Granted, the majority don't seem to be, at least initially in the time that you were able to study them, but some of these could be really significant. So I wonder, just going forward, from your perspective, are there any unresolved questions, things that you would like to see studied further as we move ahead with this?

Brenda: Well, I guess I'd like to answer the question whether I really need to do an antiglobulin crossmatch. I think we don't know the frequency, although we've had some studies done with solid phase, but is the frequency of AUS the same using different antibody detection methods, or in different populations? I mean, this was a single center study, and as I said, we're a tertiary academic center which has a different population than the community. I really don't know what the current practices are for other people, what other people do when AUS is present. And, should we standardize this? Is there something we should be doing? And, again, I would love to come up with a way to determine whether these AUSs

actually represent clinically significant antibodies and have some sort of standardized workup.

- Joe:** Yeah, to get that crystal ball going, right? To have something that just goes, "Bam! Oh, this one, this one is much more likely to be significant. Let's go down this pathway." That would be great.
- Brenda:** It would be. And I was hoping that's what we would find, but as with a lot of studies, you don't get what you want.
- Joe:** Well, so Brenda, before I let you go, I wonder if we could swing back around to your case? You had described to us the case in the beginning of a 96-year-old lady who came in for a valve replacement, who had the perfect description of what you found, an AUS. She got transfused multiple units (I can't remember how many you said) of crossmatch-compatible red cells. And I'm wondering, did you have any followup on her case?
- Brenda:** Yes. She got five units of crossmatch-compatible red cells on the day of her procedure. And then, four days later, we got a new sample, and in that sample, she had a positive antibody screen, and we were able to identify an anti-E.
- Joe:** Uh-oh!
- Brenda:** "Uh-oh is right!" Luckily, only 30% of those units were probably positive. So she didn't have too much trouble. I don't recall her having any problems. So, I'm sure she eventually cleared those cells, if we gave her E-positive cells.
- Joe:** Well, that is such a great illustration, and I'm sure you're happy that you gave her crossmatch-compatible cells, but as you said, if the anti-E wasn't necessarily showing then, that could have been, could have potentially been an issue. I'm glad to hear that it wasn't for her. That's good news.
- Brenda:** Well, we definitely ruled it out on the initial sample and it was definitely there on the next sample.
- Joe:** That's amazing, four days later that could happen. I mean that would suggest, wouldn't it, that that was probably an antibody that was there before, like an evanescence situation?
- Brenda:** Absolutely.
- Joe:** Yeah. Sounds like it. Well, Brenda, this has been a great look at this. As I said before, it can be a frustrating and difficult and challenging situation for blood banks. Clinicians don't like hearing, "There's something there. We're not sure what it is, if it means anything at all," but I think that you've helped us kind of see the big picture of this a little more, and I really appreciate

the look at it that you've taken with us. I wonder, is there, is there anything else you'd like to leave us with before we go?

Brenda: I guess I want to reiterate that antibodies of undetermined specificity are common findings and really they should be taken seriously because, as we've shown, some of them do turn into clinically significant antibodies, and I would love for someone to take a different look at this to see if they can come up with a way to differentiate those that are clinically significant from those that are just nuisance reactions.

Joe: All right, podcast listeners, you have your marching orders there! Dr Brenda Grossman is asking you guys to figure this out. So there you go. So I just, maybe the 10 or 15 people that listen to this podcast are going to do that. Brenda, that's what's going happen.

Brenda: It's more than 10 or 15.

Joe: Slightly, maybe slightly. Alright, Brenda well, thank you again so much for being with me here on the podcast. I really, really appreciate your time. Thanks for being here.

Brenda: Thank you. It was a pleasure.

Joe: Hi, this is Joe with a couple of closing thoughts.

Remember, you can find references and other good stuff on the show page for this episode, that's BBGuy.org/057. Also on the Blood Bank Guy site, you can find other interviews including the most recent episode, which was the interview on "Transfusion in Liver Disease" with Dr. Jeannie Callum (that one has proven to be VERY popular!) You can listen directly on the website, on Apple Podcasts, Google Play, Stitcher, Spotify, or wherever you get your podcasts. Speaking of that, if you have a chance, I'd really appreciate it if you'd go to Apple Podcasts and give this podcast a review. It really helps to get the podcast out in front of more people.

I've got another episode coming very soon, in which I discuss the surprisingly high risk of a patient getting a blood clot after they get a red cell transfusion. This is a risk that is really not widely appreciated, especially among our clinician friends, and Dr. Ruchika Goel from the Simmons Cancer Institute at Southern Illinois University, the Mississippi Valley Regional Blood Center, and Johns Hopkins University, will help us understand that this association is not only real, it is a real problem!

But until that time, 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. I'll catch you next time on the Blood Bank Guy Essentials Podcast.